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NATURE

No. 4696 SATURDAY, OCTOBER 31, 1959 Vol. 184

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TAXATION OF LEARNED AND PROFESSIONAL SOCIETIES IN BRITAIN

DURING the past two decades, the professional and learned societies have encountered increasing financial difficulties. The major institutions no longer possess the intimate and social character that once was theirs, and this may well be one factor that has encouraged the proliferation of specialist societies with only a limited range of interests. Nevertheless, even those with a membership of several thousands have encountered difficulties in maintaining the publication of professional periodicals, and this is a matter to which the Nuffield Foundation has given some attention since 1955, in co-operation with the Royal Society for scientific periodicals, and the British Academy for periodicals in the humanities. Moreover, there appears to be a marked reluctance on the part of younger scientists and technologists to join the more general societies; the tendency is to support institutions the membership of which constitutes a professional qualification.

It is probably too early to assess as yet the effect of the concession made in the Finance Act last year which allowed fees and subscriptions to professional bodies, learned societies, etc., not of a mainly local character to be claimed as expenses against income tax. When this clause was considered in the House of Commons on June 17, 1958, the Financial Secretary to the Treasury explained that while there was no logical reason to exclude the local societies, to include them would place an administrative burden on the Inland Revenue out of all proportion to the relief to the taxpayer. It was estimated that the national societies alone probably numbered about 3,000, and since the subscriptions to the local societies were usually small, the cost to the general body of taxpayers was not consonant with any relief to the individual.

During the past year, the Inland Revenue has examined the activities of the national societies in the light of the new clause, and most of the societies have already been able to notify their members that they are in a position to claim relief. If it could be shown that the membership of these societies has appreciably benefited from this concession, it might be reasonable to re-open the question of the local societies and, with the position of the national societies already clarified, the Inland Revenue should not find it an unreasonable task to deal with the local learned societies.

To the professional and learned societies, however, the effect of this concession is unlikely to be great: its main benefit is to the individual member. The concession is important, so far as the societies are concerned, in helping to offset a number of adverse factors against which most of them have been struggling: stationary or declining membership (in spite of the increasing numbers of scientists and technologists), mounting costs of printing and publication, and the steady rise in postal charges. Some of these factors could be offset by a measure of rationalization, and the pilot survey of the publishing and distribution practices of the learned periodicals carried out by Mr. Robert Lusty on behalf of the Nuffield Foundation may be regarded as a step in this direction.

A much more serious matter for the learned societies and professional institutions is, however, the complexity of rulings about the payments they make to local authorities in the form of rates. During the past three years or more, the Parliamentary and Scientific Committee has made repeated representations to the Government on this matter, submitting, for example, a comprehensive memorandum to the Minister of Housing and Local Government directing attention to anomalies in the rating of both scientific societies and research associations. During the second reading debate of the Local Government Bill in the House of Commons on December 9, 1957, the Minister announced that he intended to set up a committee to give special consideration to the rating of charities and similar organizations, and the chairman of the Parliamentary and Scientific Committee was eventually informed that the terms of reference of this committee would be wide enough to permit consideration of the special position of scientific institutions.

This committee was, in fact, appointed on January 22, 1958, under the chairmanship of Sir Fred Pritchard, "To review the present treatment for rating of hereditaments in England and Wales occupied for purposes of a charitable nature or for other similar purposes (other than hereditaments to which Section 7 of the Rating and Valuation (Miscellaneous Provisions) Act, 1955, applies); to consider in particular the provisions of Section 8 of the Act of 1955 and of the Scientific Societies Act, 1843; and to advise on the proper treatment for rating of the hereditaments within these terms of reference". The Parliamentary and Scientific Committee submitted a memorandum on the general lines of that previously submitted to the Minister of Housing and Local Government and was later invited to submit oral evidence: only the Royal Society also gave oral evidence.

The report of this Committee*, which was presented to Parliament in August 1959, is of considerable general scientific interest, quite apart from the specific recommendations and their effect on scientific or professional societies. It should be remembered that rates are the main source of revenue within the direct control of local authorities, and it is the occupier rather than the owner of a hereditament

* Report of the Committee on the Rating of Charities and Kindred Bodies. Pp. iv+86. (Cmnd. 831.) (London: H.M. Stationery Office, 1959.) 5s. net.

who is ratable in respect of it. The earliest general enactment still extant which exempts a specific class of hereditament from rates is Section 1 of the Scientific Societies Act, 1843, which covers societies instituted for the purposes of science, literature or the fine arts. This section exempts such societies from rates if these societies are supported wholly or in part by annual voluntary subscriptions and do not make any dividend or bonus to their members. It would seem that in granting such exemption, Parliament intended to do no more than put the buildings of such societies on a par with buildings dedicated to public purposes.

The commonest ground for loss or refusal of exemption has been failure to comply with the condition about support by annual voluntary contributions, and in reviewing the working of the Act, the Committee gives most attention to this condition, which has led to a good deal of litigation and is still not entirely clear. It appears that income from invested voluntary contributions is not itself an annual voluntary contribution and, in consequence, a society which depends almost entirely upon voluntary contributions but accumulates trust funds for prizes, exhibitions or scholarships could ultimately be disqualified because the investment income from contributions, although undoubtedly voluntary, was too large in relation to current activities. Moreover, the Committee could often see no difference between societies within and those without the exemption, and several societies now exempt appear to be fundamentally different from the kind of institution which members of Parliament who supported the Bill in 1843 appear to have had in mind.

The Committee was agreed that the provision could not be left unchanged; and because wherever the line were drawn there would be anomalies at the margin and any re-drafting of the conditions precedent to exemption would give rise to fresh litigation, the Committee invited the Royal Society and the Parliamentary and Scientific Committee to give oral evidence. The Committee was not, however, satisfied that any of the societies had a better claim to exemption than other charities. Most of them would be entitled under the Committee's other recommendations to 50 per cent mandatory relief as charities and, accordingly, the Committee recommended that the Scientific Societies Act of 1843 should be repealed.

Nevertheless, the Committee, like the Sorn Committee which reported in September 1954 on the Scottish rating system, recognizes that to withdraw abruptly the exemption under the Scientific Societies Act, 1843, might cause undue disturbance to the finances of the bodies concerned. It suggests, therefore, that in the first full financial year after the repeal of the provision, none of the societies which was a beneficiary under it at the date of repeal should be liable for rates in respect of hereditaments which were exempt at that date. In the second year, the rates which, apart from the transitional arrangements, would have been payable by the societies concerned should be abated by four-fifths, and in the third year by three-fifths, in the fourth year by two-fifths, and in the fifth year by one-fifth. A scientific society would thus not be liable for the full 50 per cent until the sixth and subsequent years, while if it was not a charity it would pay 20 per cent in the second and 40 per cent in the third, rising to full rates in the sixth and subsequent years.

The Committee shares the general view of its witnesses that Section 8 of the Rating and Valuation (Miscellaneous Provisions) Act, 1955, which governs the rates payable by charitable and other organizations, is unacceptable as a permanent provision, and it sees no justification for giving permanent rate relief to all organizations in so wide a field. It considers that the time has come to introduce a measure of uniformity and certainty into the rating relief enjoyed by bodies within its terms of reference, and that a satisfactory scheme should be simple and economical to administer and should not add materially to the rates borne by other classes of rate-payer. Its essential basis should be mandatory relief for the great majority of the classes of organization which in the past have enjoyed some measure of relief.

It recommends accordingly that charities should have mandatory relief and that, although the decision as to the amount must be to a considerable degree arbitrary, relief of 50 per cent strikes a reasonable balance. It does not consider that there is any need to re-define the term 'charity' for rating purposes only, and in the White Paper outlining its policy on Charitable Trusts in July 1955, the Government rejected proposals for a new definition for general purposes. The Committee recommends that organizations on the fringe of the field of charity should be eligible for relief at the discretion of the local authorities, but it does not recommend that charities in general should be excluded from relief on the ground that the body is national or that it is in receipt of Exchequer grant or fees or because its voluntary income is small. The position of the universities was specially considered, but the Committee does not consider that, on balance, the exclusion of all university institutions would be justified by the evidence or arguments presented.

Representations were made to the Committee that a new statutory relief of 75 per cent should be allowed to industrial research associations, and on this also oral evidence has been heard from the Parliamentary and Scientific Committee. The Pritchard Committee, however, submits reasonably enough that research associations are much more akin to the research establishments of individual firms than to charities, and that the relief considered appropriate for research establishments conducted by individual firms within the curtilage of their industrial hereditaments should be extended to the premises of industrial research associations. This view is in accordance with that expressed in evidence by the Department of Scientific and Industrial Research.

No pronouncement has yet been made by the Government regarding its acceptance or otherwise of the recommendations of the Pritchard Committee; this matter of rating relief is only one way in which Section 8 of the Rating and Valuation (Miscellaneous

Provisions) Act, 1955, has touched the scientific and other learned societies very closely. Under the Local Government Act, 1948, valuation officers of the Board of Inland Revenue became responsible for valuation rating on February 1, 1950, with the object of securing uniform standards of valuation, and this, of course, precluded the continuation of the practice of sympathetically undervaluing hereditaments occupied by charities and kindred bodies. Section 8 of the Rating and Valuation (Miscellaneous Provisions) Bill introduced in March 1955 was intended to avoid the substantial increases in liabilities for rates which such bodies would otherwise have incurred, and it was quite clear in the debates on the Bill, which received Royal Assent on July 27, 1955, that the Government fully appreciated the difficulties of the scientific and learned societies.

The Pritchard Committee in its report emphasizes that this Section of the Act provides the first statutory relief from rates for charities as such, and the first endorsement by Parliament of the relief previously given extra-statutorily by local government in various ways. Further, this element of mandatory relief was introduced by the Government not, initially, as a matter of Government policy, but in deference to the wishes of the House of Commons after a provision relying entirely upon local discretion had been criticized from all quarters. Moreover, this particular enactment was designed as a holding provision and never intended as a permanent arrangement.

These observations are important, as they indicate clearly the line which should be taken by scientific societies and professional organizations which may be unfairly affected in the new situation. It follows, moreover, that legislation to provide some reasonable permanent arrangement is not merely probable but almost inevitable, and that it is in the debates on such legislation that the needs of the scientific and learned societies and professional organizations should be clearly and effectively presented to Parliament.

When in 1950 the valuation officers of the Board of Inland Revenue assumed responsibility for valuation for rating, they did not disturb the exemptions in the old lists unless they were asked to do so by the rating authorities; but in preparing the new valuation lists which came into force on April 1, 1956, they applied strictly the provisions of the Act of 1843 as recently interpreted by the courts. In consequence, some societies which had been exempt became liable for rates on assessments based on full current rental values, and only some of those which challenged this liability in the courts succeeded. Further, the Board of Inland Revenue has suspended the income tax rebate which the learned societies enjoyed on seven-year covenants entered into by their members, and for some of them has finally withdrawn the relief.

This is the second aspect of the situation which deeply concerns the professional associations and learned societies and other bodies, and may affect some of them much more seriously than the concession made regarding subscriptions to such bodies in Clause 14 of the Finance Bill, 1958. Indeed, although the situation is complex and is still being argued between various bodies and the Inland Revenue, sometimes in the Court of Appeal, it would appear that those bodies which have lost the right to tax rebates on subscription income guaranteed from covenants are likely also to lose rating relief and the advantage of their members being able to claim subscriptions as expenses against income tax. Probably it is not too much to say that some of the societies have only been able to meet post-war costs through the rebates in covenanted subscriptions and relief from rates which they have hitherto enjoyed.

The Pritchard Committee argues lucidly and cogently for a mandatory and uniform system of rating relief. It does not suggest that all anomalies will be removed or that there should be no discretionary relief. It is difficult to refute the argument of an eminently sensible report, but before adopting the legislation to which the report points, Parliament might reasonably re-examine the fundamental question what is a charity for tax purposes, and especially the full and wide implications of what could not unfairly be described as an assault on learned societies launched by the Board of Inland Revenue. Parliament, at least, should be concerned not so much with the benefits and advantages, to particular institutions and their members, of taxation or rating relief, but with the extent to which the public interest is served by such bodies. A recent survey of leisure and learning in Bolton and Rochdale pointed to the value of such societies in the world of to-day; quite apart from their place in the dissemination of knowledge and the maintenance of professional standards, and to their need of assistance in the maintenance and equipment of premises. The place of the scientific and learned society in the world of to-day could be appropriately re-examined in the light of all the implications of the situation on which the Pritchard Committee has now reported.

KEW, PAST AND PRESENT

The Royal Botanic Gardens, Kew

By W. B. Turrill. Pp. 256+16 plates. (London: Herbert Jenkins, Ltd., 1959.) 25*s*. net.

"KEW" to botanists, horticulturists and admirers of plants the world over is the Royal Botanic Gardens, Kew, Richmond, Surrey, England. Like many other famous places or institutions, the exact day of its founding is uncertain. This summer, however, the Royal Botanic Gardens celebrated a birthday of approximately two hundred historic, profitable, eventful and beautiful years. At this occasion grateful botanists paid tribute by manuscript, letter or personal visit. Few have honoured Kew as well, though, as William Bertram Turrill does in this book. It is a devoted tribute to the Gardens the author knew and served for forty-nine years until his retirement in 1957.

"The Royal Botanic Gardens, Kew" is both a historical account of the development of the Gardens and laboratories and a detailed description of their current contents. The book is readable, but the historical portion and that of current activities of the staff are written with so much under-statement

that one longs for greater emphasis on the personality and procedures of the men who made Kew and contribute to its reputation to-day. The Royal Botanic Gardens have served as a training ground for many scientists, explorers and gardeners, but Dr. Turrill is almost reluctant in admitting the contributions of these men. Procedures, ideas and even the architecture developed at Kew were taken by students to distant lands, and one sees the influence of Kew in some aspect of every major herbarium and botanic garden of the world. A single chapter of a scant fourteen pages describes the current scientific research of nearly eighty-five people. Three pages are devoted to "Plant Introductions via Kew", and nearly half of this concerns the story of quinine. One wishes Dr. Turrill had elaborated more the credit which is due to the activities and leadership of Kew, and in basic research and practical horticulture.

The remaining chapters, particularly those on "Economic Botany and the Kew Museums", "The Greenhouses" and Kew at various seasons, charmingly describe a tour of the exhibits, living and preserved, in infinite detail. The book will serve as a guide, supplying, where appropriate, the personal exposition of a tour leader on how trees grow or manufacture food, or how plant products are used, or where particular plants can be found. One familiar with museum and garden exhibition techniques visualizes in Turrill's account both the display and the information on the labels. At the same time, the casual reader may be unaware that chapters of a basic text-book of botany have been paraphrased to present briefly and clearly the reasons for the exhibit.

A chapter on "Wild Life at Kew" exemplifies the detail of the book in relating much information, including the introduction of the American grey squirrel and its destruction, the amount of myxomatosis in the rabbits of Kew during the year 1955, the types of weeds in the lawn or the record-sized fish caught in a Kew pond and its present location. Throughout this and other chapters are the intimate stories one gets in a personally conducted, leisurely tour by a guide who knows and loves the Royal Botanic Gardens, Kew.

Sixteen excellent plates illustrate the men and women responsible for Kew's past, the present buildings, and scenes from the Gardens. Appendixes give details on the climate, the rules and regulations, the physical plant, the composition of the staff and the chronology of the curators and keepers of the Herbarium, Library and museums. A bibliography of sixty-six titles relating to Kew, an index and a grid map, referred to frequently in the text, complete the book.

As one enjoys a garden at many hours of many seasons, so I recommend to past, present and future friends of Kew a leisurely and frequent reading of this book.

Richard A. Howard

JET PROPULSION

Jets and Rockets

By A. Barker, T. R. F. Nonweiler and R. Smelt. Pp. xiv+268. (London: Chapman and Hall, Ltd., 1959.) 35*s*. net.

THE history of this book prior to publication was unusually rich, and Mr. Nonweiler recites it with relish in his candid preface. The book was begun by Mr. Smelt in 1945, and then passed to Mr. Nonweiler after the former 'went West to the States'; finally Mr. Barker brought it up to date and completed it. Although a few authentic touches of antiquity survive, most of the book has been satisfactorily modernized, and the reader need have only occasional qualms on this score.

The book is intended as an introductory text-book on all forms of jet propulsion, rocket, ramjet, turbojet, pulse-jet and various hybrids, and on the whole, it achieves this purpose well. The authors describe clearly the main features of each power plant, and preserve a fair balance; they do not delve very deeply into the specialized problems of each engine, but this cannot be expected in a 250-page book so wide in its scope. The chemistry of rocket combustion and the thermodynamics of the various engines—the theory underlying the calculation of thrust coefficient and fuel consumption—are presented in adequate detail; and the methods of calculating drag and the design of air intakes are also thoroughly discussed. One of the best features of the book is its emphasis on the close links between the internal thermodynamics of an engine and its external aerodynamics. This emphasis is particularly valuable because many text-books on propulsion tend to ignore the external aerodynamics, although minimizing drag can be just as important as maximizing thrust, especially at supersonic speeds.

Unfortunately, the book is marred by lack of attention to detail. Several of the formulæ are in error (e.g., equations 5.13 and 5.15), some of the graphs lack units, the spelling is erratic, misprints abound and there are many stylistic lapses. Chapter 15 is in places sadly out of date: it gives the impression that the V2 was the ultimate in rocket missiles and that space vehicles are virtually impossible. Indeed, throughout the book, German war-time engines, now museum-pieces, are too often quoted as examples, thus giving the false impression that the accompanying text is equally obsolete. On one point of detail the book is excellent: there are more than 120 diagrams and photographs, most of them pertinent, clear and informative.

D. G. King-Hele

NUCLEAR FUEL TREATMENT

Chemical Processing of Nuclear Fuels

By Dr. F. S. Martin and Dr. G. L. Miles. Pp. x+242. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1958.) 40*s*.; 7.50 dollars.

ALTHOUGH this book is intended mainly as an introduction to the problems of chemical processing of nuclear fuel after irradiation in a reactor, its scope extends much further. In Part 1, which deals with "Nuclear Considerations", the three main systems uranium-235, uranium-238–plutonium and thorium–uranium-233 are considered separately and the reactions occurring under thermal neutron irradiation shown diagrammatically.

The several highly developed solvent-extraction processes employed in nuclear processing are described and decontamination factors listed. The requirements of a process for purifying the plutonium product of primary separation are also enumerated and the value quoted for the overall recovery of plutonium (99·4 per cent) in one process shows how highly developed this particular technology has become.

In the middle chapters, the authors survey other processes which have been considered (and in some cases developed) for the separation of heavy nuclides from fission products. The range covered is sufficient to indicate the amazing volume of research which has been carried out on both sides of the Atlantic in this field—ion-exchange separations, metal distillations, halide volatilizations, extractions by molten metals, purification by slagging processes, extraction by fused chlorides as well as the more conventional (and historically important) processes of separation by precipitation.

There is also a section in the book dealing with the disposal of effluents and fission product recovery, a field which has received much attention from authors, both knowledgeable and otherwise, in recent years. It is sufficient to say that the treatment here is brief and chemically factual and scores on both these counts.

A criticism which may be advanced, perhaps, is that the reader is not given very clearly to understand which are the most important separation processes described. The dominant position now held by solvent extraction processes and the resultant commercial difficulties in the way of any competing technology are not brought out very fully. But such an appreciation is not necessarily a function of this book; it is abundantly clear that it represents a valuable and important contribution to chemical literature. It should find its way not only to those science and engineering graduates with some acquaintance of nuclear reactor development but also to that much wider reading public of chemists and chemical engineers who would like to read and have by them an authoritative and interesting work on the applied chemistry of nuclear power. J. E. LITTLECHILD

FOOD ANALYSIS

The Chemical Analysis of Foods and Food Products

By Dr. Morris B. Jacobs. Third edition. Pp. xxiv + 970. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1958.) 103*s*. 6*d*.

THE 970 pages of this book include more material than the title indicates. The text includes information on the make-up of several types of food. For example, the chapter on sugar foods and carbohydrates begins with a useful summary of the types of carbohydrates found in foods; the chapter on meat gives definitions of meat products and tables of typical compositions; the chapter on oils and fats contains in tabular form information on 24 fatty acids; the chapter on quality measurement includes a general introduction on flavour acceptance; while the chapter on milk comprises more than a hundred pages and includes detailed information on composition, on cheese and other products, and on adulterants.

Essentially, however, the work is a practical book for use at the bench. The book begins by describing general chemical and physical methods that are used in the analyses of food products. Directions are given for analysing constituents of all common and some less common foods. The book also includes chapters on undesirable materials in food. For example, one chapter deals with filth, and includes working directions for estimating the amount of rodent excreta, maggots, rancidity, and decomposition in fish and other foods. There are also chapters on pesticide residues, radiochemical determinations, food poisoning and preservatives. The detection of horse meat in presence of other animal tissues is discussed. Chapters are included on artificial sweetening agents and on colouring matters. Instructions are even given for such details as how to count the pits in preserved cherries from which the pits have ostensibly been removed.

In a few respects—for example, in spectrophotometry, in absence of mention of methyl cellulose, in determination of tocopherols and of carotene, in lack of reference to paper chromatography—the book is not up to date, but it would be impossible for so large a work to be kept up-to-the-minute by one author. The book has been produced in the United States and is primarily concerned with codes of practice and food laws in that country, but in nearly all cases the information has general application. The book is indeed a useful compilation.

V. H. BOOTH

A FLORA OF THE ARCTIC

Circumpolar Arctic Flora

By Nicholas Polunin. Pp. xxviii + 514. (Oxford: Clarendon Press; London: Oxford University Press, 1959.) 126*s*. net.

TWO problems immediately confront those who write on arctic plants: first, the difficulty of defining the limits of what one proposes to term 'the Arctic', and secondly, the even greater difficulty of providing an adequate and up-to-date account of that vast and virtually inaccessible area lying east of Finland and west of the Bering straits. Dr. Polunin with his extensive experience of the arctic and arctic vegetation faces up boldly to the first problem and can no doubt furnish weighty arguments in favour of what appears to be a curiously involved, indeed almost tortuous, delimitation. As regards the second problem, the author freely admits that our knowledge of the Soviet arctic is inadequate, and that, in present circumstances, no Western or American botanist can hope to compile a detailed and critical circumpolar flora. Some may feel, this being so, that any attempt to deal with the flora of the area is bound to be premature and unsatisfactory. The number of flowering plants and vascular cryptogams occurring in the Arctic is so small that the critic has some right to expect a minute and detailed analysis, and to be more exacting in his demands than if the author were attempting a survey of some tropical region with a richly diversified flora. If the reader approaches Dr. Polunin's book in this frame of mind he will find much to criticize, for the very frequent use of the tell-tale abbreviations *agg.* and *s.l.* after the scientific names shows how much has still to be done before the last word can be written on this subject. But half a loaf is better than no bread, and the less exacting will be glad that Dr. Polunin has had the energy and enterprise to give us a concise, lavishly illustrated and, for practical purposes, a tolerably complete account of these northern plants.

British botanists, whose thoughts turn not infrequently to those epochs when much of Great Britain lay buried under ice and snow, will be intrigued to see how many truly arctic species still survive from those bygone glaciations, and (bearing in mind recent

records of *Koenigia*, *Diapensia* and *Artemisia norvegica*) some may choose to ruminate on the number and likely identity of species yet to be discovered here. Palynologists, geologists and archæologists will also find, in these pages, the sort of information that can save hours of exhausting work in the identification of doubtful grains or fragments. Two features that will certainly not be commended by botanists are the absence of author citations in the main body of the text, and the invention of popular names, some of which (for example, "Boreal Blinking-chickweed") would, in a less august environment, raise a laugh. It is a pity, too, that space should have been devoted to derivations of generic names; such learning is scarcely called for in a book of this sort. The illustrations, though uneven in quality, are on the whole very pleasant to look at, and sufficiently detailed to give us a very fair idea of each plant. Printing and format are excellent, *though the regrettably high* price must necessarily put the book beyond the means of many who would be happy to possess it.

R. D. Meikle

NEW IDEAS FOR INDUSTRY

Investment in Innovation

By C. F. Carter and B. R. Williams. Pp. ix+167. (London: Oxford University Press, 1958.) 15*s.* net.

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records of *Koenigia*, *Diapensia* and *Artemisia norvegica*) some may choose to ruminate on the number and likely identity of species yet to be discovered here. Palynologists, geologists and archæologists will also find, in these pages, the sort of information that can save hours of exhausting work in the identification of doubtful grains or fragments. Two features that will certainly not be commended by botanists are the absence of author citations in the main body of the text, and the invention of popular names, some of which (for example, "Boreal Blinking-chickweed") would, in a less august environment, raise a laugh. It is a pity, too, that space should have been devoted to derivations of generic names; such learning is scarcely called for in a book of this sort. The illustrations, though uneven in quality, are on the whole very pleasant to look at, and sufficiently detailed to give us a very fair idea of each plant. Printing and format are excellent, though the regrettably high price must necessarily put the book beyond the means of many who would be happy to possess it.

R. D. Meikle

NEW IDEAS FOR INDUSTRY

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A NEW EXPERIMENTAL TEST OF SPECIAL RELATIVITY

By J. P. CEDARHOLM
I.B.M. Watson Laboratory
AND
Prof. C. H. TOWNES
Columbia University, New York

EXPERIMENTS which have tested special relativity have usually been forced to rely on great delicacy and precision in order to detect or examine the small differences between predictions of special relativity and those of alternate theories. This is because these differences appear multiplied by a very small quantity $(v/c)^2$, where c is the velocity of light and v is some relative velocity which is generally much smaller than c. While giving a clear-cut support to special relativity over some other theories such as a simple ether, experiments have not generally measured the small terms in $(v/c)^2$ with impressive fractional accuracy. Michelson and Morley's first experiment[1], for example, was of remarkable precision. But it was searching for a change in light-path of only about one part in 10^8 due to the motion of the Earth about the Sun on the basis of the then current ether theory, and was able to set an upper limit no less than 1/40 of this, or an ether drift of about one-sixth the orbital velocity of the Earth. Subsequent very refined experiments[2] of a similar type succeeded, a half-century later, in setting an upper limit on any ether drift of 1/20 the velocity of the Earth around the Sun. Others[3] even suggested the existence of an ether drift as large as about one-fifth of the orbital velocity of the Earth. The advent of very high precision atomic clocks suggests that still more exacting experimental tests may now be made; one such, which is now more or less completed, is reported here.

The experiment compares the frequencies of two maser oscillators[4] with their beams of ammonia molecules pointed in opposite directions, but both parallel to a supposed direction of motion through the ether. If both masers are rotated 180°, and their frequencies again compared, a change in relative frequency should be found due to motion of the masers through the ether, assuming the molecular vibrations are unchanged by such motion. A precision of one part in 10^{12} has been achieved in this frequency comparison, and failure to find a frequency change of the predicted type allows setting the upper limit on an ether drift as low as 1/1,000 of the orbital velocity of the Earth. This precision also provides a test for some other effects which will be discussed below.

The effect on the frequency of a beam-type maser oscillator of motion through the ether was first worked out by Møller[5]. A brief, somewhat intuitive explanation of this shift follows. In this device, ammonia molecules in an excited state travel at thermal velocities along the axis of a circular cylindrical cavity, giving it energy. If the cavity is stationary in the ether, the standing waves may be considered to be made of travelling waves with wave-fronts nearly parallel to the axis. As the molecule moves along the axis, there is then no Doppler shift. If the apparatus is moving axially through the ether at velocity v, the wave-fronts must tilt at an angle $\alpha = v/c$ in order to follow this axial velocity. Hence, molecules travelling at velocity u through the cavity produce a frequency shifted by the Doppler effect of an amount $\nu u\alpha/c = \nu uv/c^2$. Here ν is the molecular frequency. Since $uv\nu/c^2$ depends on the relative direction of u and v, two masers with oppositely directed beams should have frequencies which differ by $2uv\nu/c^2$ due to this effect. If each is rotated 180°, the total change in their frequency difference is $4uv\nu/c^2$.

A more precise derivation of this effect is obtained from the fact that special relativity predicts the same result as does an ether theory, provided that the FitzGerald contraction $\sqrt{\left(1 - \frac{V^2}{c^2}\right)}$ is introduced for any length parallel to the motion v through the ether, and also that the proper time of any clock or oscillator is modified by the same factor $\sqrt{\left(1 - \frac{V^2}{c^2}\right)}$ due to this motion. In other words, any effect due to motion through a simple ether is just compensated by appropriate changes in scale for length and time which correspond to the Lorentz transformation. If, then, an ether theory is used without FitzGerald contraction and time dilation, the expected shift in frequency may be computed from an examination of the effects of these changes of scale for length and time.

Consider first the FitzGerald contraction. Its effect on the frequency of maser oscillation is very small and may be neglected because this frequency is rather insensitive to the dimensions and resonant frequency of the cavity[4].

The time dilation, however, produces the effect we seek. If the cavity moves through the ether at a velocity v and the molecule through the cavity at velocity u, then the molecular velocity through the ether is $V = u + v$, and the molecular time will be slow, for an observer in the framework of the ether, for the factor:

$$\sqrt{\left\{1 - \frac{(u+v)^2}{c^2}\right\}} \approx 1 - \frac{u^2}{2c^2} - \frac{uv}{c^2} - \frac{v^2}{c^2}$$

But time in the actual laboratory framework, which is fixed with respect to the cavity, is slow by the factor:

$$\sqrt{\left(1 - \frac{v^2}{c^2}\right)} \approx 1 - \frac{v^2}{2c^2}$$

Hence the molecule would appear slow to an observer in the laboratory by the difference between these two, or by the factor:

$$1 - \frac{u^2}{2c^2} - \frac{uv}{c^2}$$

The first small correction is the well-known transverse Doppler effect, and is independent of ether drift. The second small correction is the discrepancy uv/c^2 which would occur if we were to accept a simple ether and no time dilation in the proper oscillation of the molecule, as postulated in Møller's original discussion[5].

The above derivation makes it clear that failure to see any change in time equivalent to the small fractional amount uv/c^2 may be explained away by the assumption of a time dilation for those who wish to adhere to an ether with such peculiarities. Hence the experiment is more closely related to the Kennedy–Thorndike experiment[6] than to that of Michelson and Morley. A null result in the latter needs, of course, only a FitzGerald contraction for an explanation in terms of an ether theory.

For performance of the present experiment, two ammonia beam masers were mounted with oppositely directed beams on a rack which rotated about a vertical axis. The frequencies of these oscillators are near 23,870 Mc./s. The thermal velocity $u = 0{\cdot}6$ km./s. for NH_3 at room temperature. If the orbital velocity of the Earth is assumed to be the rate of motion through the ether, then $v = 30$ km./s. and the frequency change $4uv\nu/c^2 = 20$ c./s. when the masers are rotated 180° from an initial east–west position at noon or midnight.

During a small fraction of a second the relative frequency of the two masers fluctuates randomly about $\frac{1}{10}$ c./s. Over somewhat longer periods, such as those required for measurement before and after rotation, the average frequency difference does not vary more than about $\frac{1}{50}$ c./s. or one part in 10^{12}. Hence the 20 c./s. variation expected on an ether theory would be very easily detected. Variation of about 1 c./s. on rotation of the two masers was in fact observed. However, this variation could be eliminated by magnetically shielding the masers, and without shielding it remained constant to within about $\frac{1}{50}$ c./s. as the Earth rotated throughout a 24-hr. run. This shows that no more than about $\frac{1}{50}$ c./s. shift could be attributed to an ether drift.

The experiment involving rotation of the two masers was carefully done for the first time on September 20, 1958 [7]. No proper effect as large as $\frac{1}{50}$ c./s. was found. Hence, since the orbital velocity of the Earth of 30 km./s. would have given an effect of 20 c./s., the ether drift could not have been larger than 1/1,000 of this value, or 30 m./s. It is, of course, possible for the motion of the Earth to be just cancelled by the motion of the solar system through the ether at some particular time of the year. The experiment has now been repeated at the Watson Laboratory during 24-hr. runs at approximately three-month intervals throughout the year. In none of these runs was any effect as large as $\frac{1}{50}$ c./s. found.

The present experiment sets an upper limit on an ether-drift velocity about one-fiftieth that allowed by previous experiments. This is in part because the effect measured is linear in the ether drift velocity v. An experiment of the Michelson–Morley type is designed to detect a fractional change of the form $\frac{1}{2}v^2/c^2$, which is an order of magnitude larger than the term uv/c^2 discussed here. An upper limit of 1/400 of $\frac{1}{2}v^2c^2$ has been set by the very careful experiments of Joos[2] with a Michelson interferometer. However, since this term is second order in v, the upper limit given for the ether-drift velocity is one-twentieth of the orbital velocity of the Earth, or 1·5 km./s. The present experiments have the advantage that the expected effect is linear in v, and also that two clocks can now be compared with much greater precision than can two distances. This experiment, involving a comparison of two maser oscillators to an accuracy of one part in 10^{12}, may perhaps represent the most precise experiment so far reported.

For most physicists, a confirmation of the fundamental postulate of special relativity that no absolute motion can be detected comes as no surprise, and a more precise experimental test may not even seem important because this postulate is so intuitively satisfactory and firmly accepted. It should be noted, however, that the positive detection of an effect in the present experiment could give some new information without necessarily contradicting the general principles of relativity. The motion of the Earth involves velocity relative to other parts of the solar system, as well as to the fixed stars and external galaxies. Hence this relative motion might, in principle, produce some anisotropy in space and some shift in relative frequency of the two masers when they are rotated by 180°.

Dicke[8] has suggested that an effect due to motion with respect to fixed masses in the universe should be present which is of the order of the fine structure constant, α, times the effect due to ether drift. This would correspond to a frequency shift in the present experiment of the order of $\frac{1}{7}$ c./s. Reasons given by Dicke why such a shift might occur are speculative, but very interesting. The present results allow no shift larger than $\frac{1}{50}$ c./s., which gives some indication against a term of the order $4\alpha uv\nu/c^2$.

Optical maser oscillators[9] should also lend themselves to interesting experiments on relativity, since they will probably be capable of examining changes in length as small as one part in 10^{12}. An optical maser oscillator could be constructed with a resonance between two étalon plates which is narrower in frequency than the atomic resonance supplying energy. In this case the frequency would depend primarily on the spacing between the plates, rather than on the atomic frequency. It is estimated that the oscillation would be monochromatic to about one part in 10^{11}. This suggests an experiment in which the oscillations of two optical masers are beat together in a photocell. One of the masers may be rotated about a vertical axis. On the basis of an ether theory, the beat frequency should then vary by an amount $\pm v^2\nu/2c^2$, for the same reasons that the Michelson–Morley experiment was expected to show a variation of path length. The fraction v^2/c^2 is 10^{-8}, so that its presence could probably be tested with excellent precision.

[1] Michelson, A. A., and Morley, E. W., *Amer. J. Sci.*, **34**, 333 (1887).
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ECHO-LOCATION AMONG *COLLOCALIA*

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IT is known that a number of species of the swiftlets (*Collocalia*) of south-east Asia are able to fly in total darkness in the caves in which they nest. When on the wing in the dark or in poor light they utter a series of click-like calls in very rapid succession so that the final effect is a staccato rattle. Novick[1] has recently demonstrated that this call is essential for oriented flight in darkness by *Collocalia brevirostris unicolor*, a swiftlet found in Ceylon.

A similar call is heard from *Collocalia maxima lowi*[2], which nests in a number of caves in Sarawak[3]. In 1957, recordings of this swiftlet were made for the Sarawak Museum with the assistance of staff of Radio Sarawak. Successful recordings were made both under natural conditions in Meraja cave, Bau[4], known to be inhabited only by *C. maxima*, and of individuals of the same species flying singly in the dark-room of the Sarawak Museum, which measures 11 ft. by 16 ft. by 12 ft. high and has interior walls finished in rough plaster and distemper.

The recording apparatus used was a Philips hand microphone type 9564/10 with an E.M.I. portable battery-operated recorder, type *L2B*. Parts of the tape were later played into the 'Sonograph' sound spectrograph to give a plot of frequency spectrum against time. They were also played back into an oscillograph which was photographed on moving film. From these films the spectrum was calculated by carrying out a Fourier transform of the waveform.

Although blindfolding and deafening experiments were not performed, there are several features of the rattle call of this swiftlet that emphasize its function in dark orientation; many of those listed below are discernible on the tape recording. (Copies of the edited tape, with commentary, are held in Kuching, at Cambridge and by myself.)

(1) Birds approaching the cave from outside are heard rattling while still some distance from the mouth, but those leaving the cave by day in direct flight are silent well within it when in sight of the mouth. This has already been noted and discussed by Novick (*op. cit.*). By night, however, outgoing birds continue to rattle beyond the mouth.

(2) In imperfect darkness the rattle is not continuous but intermittent; clearly its use is not obligatory and, for example, in the dim light near the cave mouth, it is employed only in dark corners where eyesight fails.

(3) In complete darkness the rattle is continuous, although it tends still to be somewhat spasmodic when the bird is on a familiar flight line through a large chamber. The highest rate of steady rattle recorded on the tape is six clicks per sec.; but when a bird approaches its nest site, or if it is frightened in the dark, the rate may be higher still.

(4) In dark regions of the cave away from the nest sites, where all birds are in passage to or from the mouth, only the rattle call is heard and never song or other vocalization; apparently the two types of call cannot be uttered simultaneously. This suggests that the mechanism is syringeal; however, the rattle sound is so unlike any other call that a different mechanism may be involved.

(5) Roosting or nesting birds do not utter the rattle call; a bird flushed from the nest is silent for the first yard or two of flight. For this reason attempts to record at the nest sites were unsuccessful.

(6) The rattle call appears late in development, and well-grown fledglings which are forced to fly prematurely often do so silently, and are then totally disorientated in darkness.

In the Museum dark-room, five birds were flown singly. In every case the rattle call was 'switched on' the instant the electric light was switched off; when the light was turned on again it ceased less abruptly, tending to die away slowly into short well-spaced bursts or single clicks. Such brief outbreaks of rattle are heard in the field from swiftlets flying near cliffs or mountain tops, or diving to drink (on the wing) from the rivers. Chasen[5], in an account of weather movements of mixed swift and swiftlet flocks in Malaya, records the rattle call far from any cave.

In caves the rattle is never heard from birds at roost and usually ceases the moment they alight on the nest; but occasional clicks or brief outbreaks of rattle were heard from the caged swiftlets in transit from Meraja cave to the Museum. It seems likely that in unfamiliar surroundings or when close to solid (or liquid) surfaces the rattle call may be used tentatively to supplement eyesight even in full daylight.

Analysis was applied to three recordings: a single bird flying in the cave, a single bird flying in the dark-room, and very many birds rattling simul-

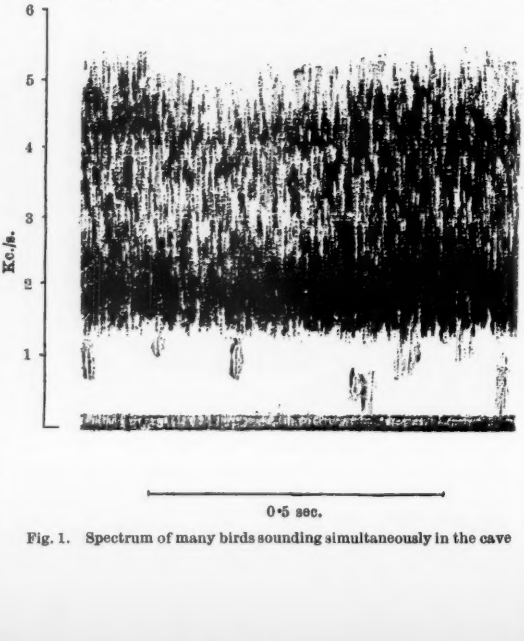

Fig. 1. Spectrum of many birds sounding simultaneously in the cave

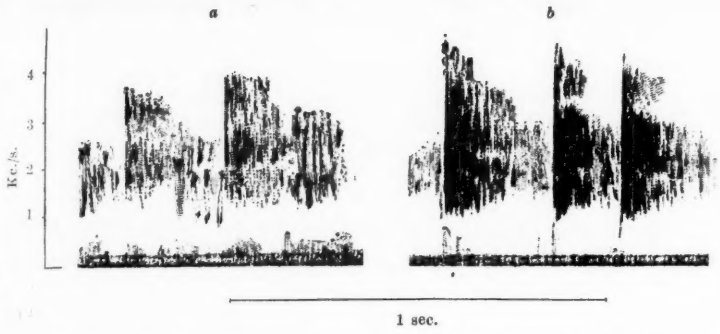

Fig. 2, *a* and *b*. Two different birds recorded separately in the museum dark-room

taneously in the cave mouth by night. Results show that the single click is very brief, lasting approximately 2·5 msec. The spectrum of the crowd of birds sounding together is continuous from 1·5 to 5·5 kc./s., with peaks at around 2 and 4·5 kc./s. (Fig. 1). Isolated bursts of sound from individual swiftlets all fall within the same frequency-range (Fig. 2), and successive clicks are more or less similar although they show no exact correspondence of wave form. The Fourier analyses of the wave-forms correspond well with the sound spectrograms. The single clicks of different birds (Fig. 2*a* and *b*), however, show different distributions of frequency peaks within the characteristic range; this variation might assist each bird to identify the echo of its own call among a crowd, although in most circumstances the coincidence in timing between emission and echo would be quite sufficient to discriminate against the calls of other birds. For example, it can be shown that if the bird neglects all echoes returning more than 10 msec. after emission (that is, reflected from more than 5 ft. away) it may be able to infer the presence of an obstacle with 90 per cent certainty, even though there may be twenty other birds within a radius of 20 ft. This calculation assumes that the echoes from only three successive clicks are noted; if a greater number can be utilized the discrimination increases correspondingly.

The only other avian genus known to echo-navigate is the oil-bird, *Steatornis*[6]. This utters a similar call; but the mean frequency is considerably higher (7·3 kc./s.) and the range (6–10 kc./s.) does not overlap with that of *Collocalia maxima*, which is shown to rely for sonic-navigation on lower frequencies than any other bird, bat or dolphin so far investigated. Other *Collocalia* in Sarawak also utter the same call; one species (*C. esculenta*) lacks it. As part of a survey of the life and habits of the genus in Borneo at present in hand, it is hoped soon to extend investigation of the use of echo-navigation and of the physiology of the mechanism involved.

I am grateful to Mr. Tom Harrisson, curator of the Sarawak Museum, for the opportunity to work in Sarawak, for encouragement in the study of *Collocalia* and for criticism of this communication before publication; to the staff of Radio Sarawak, whose interest and help made these recordings possible; and to Dr. K. E. Machin, of the Department of Zoology, Cambridge, for the analysis of the recordings and for the discussion on the identification of a bird's own call.

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IONIZATION PHENOMENA IN GASES

THE fourth International Conference on Ionization Phenomena in Gases took place at Uppsala, Sweden, during August 17–21. The very wide programme included sections on plasma physics, as well as on fundamental processes and other applications of electric discharges, and attracted a representative gathering of about 800 scientists from research organizations active in this field in twenty-five different countries. This number of delegates represented a considerable increase when compared with the previous Conference at Venice in 1957, and reflects the increasing world interest in this subject. After a short opening address by the honorary president, the Rector Magnificus of the University of Uppsala, Prof. Torgny Segerstedt, the general pattern of the Conference followed closely that of previous conferences in the series[1], in that part of each day was devoted to plenary sessions, at which general survey papers were read, while for the rest of the time the Conference split into four parallel sessions taking place simultaneously. The titles of these sessions were: (1) Fundamental Processes; (2) Different Types of Discharges and Their Application; (3) Theoretical and Experimental Studies in Plasma Physics; and (4) Production, Confinement and Heating of Plasmas. Since there were, altogether, about 250 papers presented, considerations of space make it impossible to give a complete coverage in this report; instead, a selective review of the papers given in the plenary session together with those in the related sessions which can be regarded as having the most general interest is given, but this inevitably means the omission of mention of many papers.

Fundamental Processes

W. L. Fite (San Diego) presented a paper surveying recent advances in the study of collision processes in gases, in which he first discussed the results obtained for the cross-section for scattering and electron exchange using modulated crossed-beam techniques, work with which he himself has been associated for a

number of years. Further work on the determination of inelastic collision cross-sections using the trapped electron method was then discussed, and the paper concluded with a review of recent results on heavy-particle collisions using an extension of the modulated cross-beam techniques. This particular aspect of the work was discussed in more detail by the same author in a later paper given in Section 1, results being given for cross-sections of charge transfer and ionization for H^+ and H_2^+, and for charge transfer and electron stripping for H^- in H within a range of ion energies from 50 eV. to 50 keV. Other experiments using crossed beams were described by R. L. F. Boyd and A. Boksenberg (London), who reported on measurements of ionization cross-section of electrons in H together with a trochoidal mass spectrometer analysis of the ions produced. The trapped electron method was also described in detail in a later paper by G. J. Schulz (Pittsburgh), who showed that using this method it is possible to study the shape of an excitation function from its onset to 3 eV. above onset. An interesting result obtained in these experiments was that there is a large inelastic peak in nitrogen and carbon monoxide at 2·3 eV. and 1·7 eV. respectively; the formation and subsequent decay of temporary negative-ion states of nitrogen and carbon monoxide was put forward in explanation of these results.

Several papers were presented on the measurements and calculation of collision cross-sections for particles of high energies up to 200 keV. Particularly active in this field are the members of the Physico Technical Institute of the Academy of Science of the U.S.S.R., who presented three papers (two of which were read by N. V. Fedorenko and the other by V. M. Dukelsky), giving the results of measurement on electron-capture by multiply charged ions, on the ionization of inert gases by protons, and on the ionization of high-velocity alkali atoms by collisions with atoms of inert gases. The results of recent measurements on the growth of pre-breakdown ionization currents for several organic vapours were reported by A. E. D. Heylen (London), and for air by A. N. Prasad and J. D. Craggs (Liverpool) and by J. Dutton, F. Llewellyn-Jones and R. W. Palmer (Swansea); in the latter two papers the influence of attachment on the growth of ionization was also assessed. A technique for the measurement of attachment coefficients for very low-energy (nearly thermal) electrons in oxygen and nitrogen was described by M. A. Biondi (Pittsburgh) and the results analysed in terms of two- and three-body collisions. Other papers concerned with ionization coefficients were those of J. M. Meek (Liverpool), who put forward the proposal that the decrease in secondary ionization with increasing concentration of water vapour in air is due to a reduction of the photo-electric emission consequent on the absorption of photons by the water vapour, and of D. E. Davies, J. G. C. Milne and J. Myatt (Keele), who discussed the influence of gas purity, tube geometry and method of calculation on the determination of ionization coefficients in hydrogen and the inert gases.

Another survey paper dealing with fundamental processes was given at one of the plenary sessions by G. L. Weissler (Los Angeles), who described the techniques used to study the interaction of the vacuum ultra-violet photons ($h\nu$ = 7 to 30 eV.) with gases. Of particular interest were the results obtained using mass spectrometer techniques for identification of the ions in the determination of specific photo-ionization cross-sections, and of ion molecular reaction rates for such complex ions as N_2H^+. It was shown that with equal concentration of nitrogen and hydrogen the number of N_2H^+ ions produced per unit light intensity increased with increasing energy of the irradiating photons throughout the range investigated, whereas the number of H_3^+ ions showed a peak within this range. Further studies of the interaction of photons with gases were described in papers by D. K. Davies and F. Llewellyn-Jones (Swansea), who discussed the influence of collisions of the second kind between excited and neutral molecules, on secondary ionization in hydrogen, and by A. Przybylski (Hamburg), who reported measurements of the absorption coefficients in oxygen and nitrogen of gas-ionizing radiation produced by corona discharges in the same gases. The complex structure of the optical excitation functions revealed in experiments with mercury and helium were described by H. M. Jongerius (Utrecht) in a joint paper with C. Smit and J. A. Smit, and theoretical calculation of the 1*s*–2*p* electron excitation cross-section of atomic hydrogen was reported by A. Kingston, B. L. Moiseiwitsch and B. Skinner (Belfast).

Another topic surveyed in a paper at one of the plenary sessions was that of recent work on the study of current growth and on avalanches of large amplification, by H. Raether (Hamburg). The techniques developed at Hamburg for the study of single electron avalanches in gases such as methane (where large amplifications can occur during the first electron transit time) were described. It was reported that the probability of these large avalanches leading to *kanal* breakdown increased with the number of carriers (electrons) in the avalanche. The statistical nature of such avalanches was discussed, and in a later paper H. Schlumbohm (Hamburg) described in detail the way in which the primary ionization coefficient may be obtained from measurements of avalanche statistics, and gave results for various organic vapours. Another member of the same group, L. Frommhold, presented results of measurement of the drift velocity and mean energy of agitation of electrons, obtained by studying the electron component of electron avalanches. Raether concluded his paper by discussing the growth of ionization in the case where there is a relatively strong external illumination of the cathode, which makes statistical variations of avalanche size relatively unimportant. Experimental results for temporal growth of ionization under these conditions were given by F. Llewellyn-Jones and E. Jones (Swansea), who showed the importance of the stability of the cathode surface in such investigations which involve accurate measurements of formative time lags, and how the magnitude of the individual secondary ionization coefficients in hydrogen and hence the growth times depend on the state of the cathode surface. Another paper on the effect of the state of the cathode on growth times and sparking potentials in hydrogen was given by B. J. Hopkins (Keele) in a paper with D. E. Davies, R. K. Fitch and C. F. Gozna. Breakdown potential measurements for the interesting region below the Paschen minimum and for a.c. fields were described and discussed by H. Niesters and L. Graf (Aachen), respectively. The way in which space charge influences the current growth to give the observed voltage-current density characteristics was investigated in a paper by A. L. Ward and M. J. Reddan (Washington).

The remaining papers under this heading were mainly devoted to problems associated with the bom-

bardment of surfaces by ions and with the consequent sputtering.

Arc, Spark and Glow Discharges

Discussing recent progress in arc research in a paper to one of the plenary sessions, W. Lochte-Holtgreven (Kiel) first described results of mass spectrographic analyses of heavy ions (produced, for example, from pumping oil) in electro-magnetically compressed arcs, showing that a resolution of 1 part in 4,000 is attainable.

The way in which radiation from arc discharges has been analysed to determine the contribution due to the formation of negative ions was outlined, and it was shown that this process contributed in very large measure to the total radiation in oxygen. Similar studies in nitrogen have demonstrated the existence of a metastable negative ion of nitrogen with a short life-time of about 10^{-6} to 10^{-7} sec., which is consequently not detectable by the usual mass spectroscopic techniques ; this result is in agreement with the results of Schulz mentioned above.

Also discussed were chemical processes occurring in arc discharges, and the use of arc discharges in the separation of isotopes of neon, and of neon and nitrogen by thermal diffusion techniques.

Experimental and theoretical studies on the decay of interrupted arcs were discussed in papers by H. Edels and by D. Whittaker (Liverpool), respectively. It was shown experimentally that the initial decay of gas temperature during the first 500 μsec. is of the order of 1·7° K. per μsec. from an initial value of 7,200° K., the full recovery taking approximately 1 sec. The theoretical treatment proceeded by first determining the steady-state radial distribution for a specified current and field strength and then calculating changes in that distribution as a function of time, using the variation of thermal conductivity, specific heat and gas density with temperature. A paper on the re-ignition of a.c. arcs was given by W. Rieder and H. Schneider (Vienna), who discussed the measurement, by calorimetric means, of the cooling effect of the electrodes ; and put forward an explanation for the sudden step in the re-ignition voltage in the recovery curves for low-current arcs, in terms of the disappearance of electrons from the gap. The post-arc behaviour of two practical circuit breakers was discussed in a paper by V. K. Bisht, G. A. W. Rutgers and D. Th. J. ter Horst (Arnhem) in which measurements on the effect of pressure and rate of rise of re-striking voltage on the value of the post-arc current were reported.

The behaviour of argon arcs in axial magnetic fields was discussed in a paper by M. McChesney, P. C. McNeill and J. J. Matthews (Rugby), who gave an account of spectroscopic, rotating mirror and voltage-current measurements. Arcs in transverse magnetic fields were the subject of a paper by P. E. Secker (London), who reported investigations of the effect of the cathode surface on arcs moving in the Amperian direction ; experiments on the Amperian and retrograde motion of arcs in a magnetic field inclined to the plane of the cathode were reported in a paper by A. E. Robson (Harwell).

Sparks in long gaps were discussed in two papers by R. T. Waters and R. E. Jones, members of the Associated Electrical Industries, Aldermaston. The first paper described how the impulse breakdown of long inhomogeneous field gaps was studied by means of time lag measurements, and how attempts to explain these measurements were made by correlation with field distortion produced by space charge created in the gap by the initial corona bursts. In the second paper a different technique for studying the same problems was described. This technique involved pulse chopping and high-speed photography to measure the growth times of leader channels to within ± 0·05 μsec. The step-lengths of the spark leaders so measured were discussed in relation to the Schonland theory of lightning stepping. An analysis of the relation between lightning discharges and whistlers was given in a paper by H. Norinder (Uppsala).

Striations in the positive column of glow discharges were discussed in papers by M. Klerk (Eindhoven), who described measurement on d.c. and high-frequency discharges in tubes of various geometries, by W. Schallreuter (Greifswald), who investigated the dependence of frequency, wave-length and velocity of moving striations on various discharge parameters, and by A. Rutscher of the same laboratory, who described investigations of spectral emission from moving striations in neon glow discharges. A theoretical discussion of the positive column of low-pressure mercury-rare gas discharges, using the cross-section curves for excitation and ionization of mercury, the Ramsauer cross-section curves of the rare gases and taking into account the imprisonment of the resonance radiation was given by M. A. Cayless (Rugby). D. Müller-Hillebrand (Uppsala) described investigations of the conditions under which hydrogen–air and propane–air mixtures can be ignited by corona discharges, and concluded that discharges from points with currents up to 100 μamp. do not ignite gas–air mixtures, whereas discharges from rounded objects can ignite at an effective current of less than 5 μamp. A paper by J. S. T. Looms (Leatherhead) described investigations to assess the importance of corona as a source of interference with micro-wave communication. It was found that even when d.c. powers as great as 60 watts were developed in discharge systems 15 cm. in length, no noise output greater than 1 per cent of that of an argon noise reference source was observed. From this and other results it was concluded that d.c. corona is not a serious source of interference in the *X*-band.

Many of the remaining papers in this section were concerned with the mechanism of Geiger-counter discharges, a subject which still provokes attention.

Plasma Physics

In reviewing that section of the Conference devoted to plasma physics, main attention must be directed to the plenary session on recent results from research on controlled thermonuclear reactions.

The session opened with a series of papers from the United States ; J. L. Tuck (Los Alamos) first discussed recent work on *Scylla* and stated that it was believed that neutrons obtained from this device were truly thermonuclear. He also discussed the difficulties inherent in research on controlled thermonuclear reactions due to the onset of cyclotron resonance, and described a new machine, known as *Picket Fence*, having a four-cusped magnetic field configuration, which, it was hoped, would succeed in overcoming the problems created by cyclotron resonance. Other work on *Scylla* and on a rotating plasma containment device called *Ixion* had earlier been presented by F. L. Ribe (Los Alamos), in which measurements of

the diamagnetism of the plasma during its acceleration and after removal of the radial electric field were discussed.

R. F. Post (Livermore) reviewed recent progress on mirror machines where investigations have been concerned primarily with (*a*) studies of injection methods, (*b*) investigation of diffusion and non-adiabatic loss processes, and (*c*) further attempts to analyse the energy spectrum of trapped and heated plasma particles. The experimental difficulties being encountered at this stage in the project were outlined. Results were given of measurements of the radial distribution and rate of diffusion loss of the high-energy electron component of a heated plasma, produced by magnetic compression.

The present state of the *DCX* experiment was reported by A. H. Snell (Oak Ridge) and difficulties being encountered in providing a suitably energetic injection system were analysed. The paper discussed, among other things, observations on the spreading of the trapped ions, life-times of trapped particles, the density of the trapped plasma and an assessment of the factors that may be limiting this density. Another, earlier paper by J. S. Luce (Oak Ridge) had discussed in more detail the trapping of high-energy ions within the walls of a hollow vacuum arc. This wall prevents neutral atoms from reaching the trapped ions and therefore reduces charge exchange losses. New arc techniques were described which include plans for 15 in. diameter discharges.

Contributions from the United Kingdom were presented by G. Francis (Harwell), G. B. F. Niblett (Atomic Weapons Research Establishment, Aldermaston) and D. R. Chick (Associated Electrical Industries, Aldermaston). Progress reported from Harwell included the recent identification of Alfvén waves in a high-current toroidal discharge; an earlier paper by D. F. Jephcott (Harwell) had discussed measurements of the velocity and damping of these waves. Other fields of investigation have included experiments with linear pinch and inverse pinch systems (which have shown that in the inverse pinch case the current sheath remains stable for a longer time than in the straightforward pinch system) and some further work on *Zeta*.

Much of the work on devices such as the thetatron (azimuthal current) carried out at the Atomic Weapons Research Establishment, Aldermaston, had been reported in other papers, so that the review by G. B. F. Niblett was confined to a discussion of attempts to produce very large rates of current growth, by the design of very low inductance systems, and some very recent studies of dissociation phenomena in the hydrogen molecule. Rates of current rise of 6×10^{12} amp./sec., with peak currents of 3×10^6 amp., were reported for the parallel spark gap condenser bank known as *Maggie*. Again, because of many earlier papers from other members of the A.E.I. team, the review by D. R. Chick was confined to a description of the design and engineering of the machine to be known as *Sceptre IV*, and an outline of the proposed experimental programme.

Although members of the U.S.S.R. delegation delivered papers during this plenary session, there were no review papers in the sense of those presented by the United States and the United Kingdom. Because of this, the papers from the U.S.S.R. will be discussed in those sections of this report to which they were directly related. Two other papers concerned with thermonuclear investigations were presented in plenary sessions. W. B. Thompson (Harwell) considered fine-scale magneto-hydrodynamic behaviour in plasmas, where the effects of the finite ion Larmor radius may be important, by use of the collision-free Boltzmann equation. A consistent series expansion of this equation was used to derive first-order magneto-hydrodynamics, and the magneto-hydrodynamic shock was studied as an application of this technique. "Recent Progress in Shock Wave Research" was the title of a paper by A. C. Kolb (Washington), and in it he described the spectroscopic study of temperature and density in shock-wave fronts. Very high-ionization densities had been achieved and there was strong evidence for high temperatures, and thus considerable ionization, ahead of the travelling wave-front, probably produced by a radiation process. In the general paper sessions, those concerned with plasma physics were divided into two: (*a*) theoretical and experimental studies in plasma physics, and (*b*) production, confinement and heating of plasmas. These two sections will be briefly reviewed individually.

Theoretical and Experimental Studies in Plasma Physics

The topics in this section were transport phenomena, interactions involving electric and magnetic fields, micro-wave radiation measurements and spectra from plasmas.

An interesting theoretical approach to transport phenomena was described in a paper by M. N. Rosenbluth and N. Rostoker (San Diego), where, in a fully ionized plasma, all field particles are considered to be in equilibrium except for one 'test' particle. The resultant reaction on this test particle, due to its interaction with the field particles, consists of a frictional drag and a random force that produces acceleration and diffusion in velocity space. A systematic procedure for determining these effects with no magnetic field and in the presence of a constant magnetic field has been developed.

The topic "Interaction involving Electric and Magnetic Fields" produced the largest number of papers of any at the Conference. Among them was a paper by Dermikhanov, Gevarkov and Popov (Moscow) on "The Interaction of a Beam of Charged Particles with a Plasma". This paper described the investigation of plasma oscillations created by a continuously injected electron beam. It was shown that the maximum intensity of plasma oscillation is produced when the electron beam passes through the plasma. Electromagnetic fields with the same plasma frequencies were also found outside the plasma column; the intensity of these oscillations as a function of the density of the plasma was investigated and found to be the same as that inside the plasma column. Detection of these oscillations was possible because of the considerably increased sensitivity of the recording apparatus compared with that used by previous workers. Another paper on this topic was that by Khazchenko and others (Moscow); in this case an electron beam was modulated by oscillations in a plasma through which it passed and afterwards detected in a resonant cavity.

Other papers of interest included one by J. A. Wesson (Associated Electrical Industries, Aldermaston) on the effect of runaway electrons on the heating of a plasma, where it was shown that, for

constant electric field, runaway electrons set a limit to the temperature which can be efficiently achieved by ohmic heating ; but for constant current density, if the fraction of the current carried by the runaways is small, this fraction will decrease as the temperature increases. A paper by I. B. Bernstein and I. N. Rabinowitz (Princeton) considered the velocity distribution of plasma electrons in an external magnetic field when the ions are assumed to be infinitely massive, and electron–electron interaction is suppressed. These assumptions produced equations which could be solved numerically on a computer, and results indicated that, with an initially Maxwellian distribution, the distribution functions do not develop the double-humped character which oscillation theory indicates to be unstable.

J. E. Allen and F. Magistrelli (Rome) described experiments on the plasma sheath transition in the presence of a magnetic field. Using an azimuthal magnetic field which could be applied in the 'pinch' or 'anti-pinch' direction, they showed that a magnetic field in the pinch direction reduced the directed energy of the positive ions leaving the plasma and a magnetic field in the anti-pinch direction increased this energy. The result was shown to be in accord with theoretical predictions.

S. C. Brown (Massachusetts) in his review paper on "High Frequency Waves in Ionized Gases" considered the various types of electromagnetic waves that are set up in an ionized gas due to the application of a magnetic field. There are six natural frequencies, three being cyclotron frequencies and three plasma frequencies. Because the magnetic field is a vector the resultant oscillations in the plasma can be parallel or perpendicular to the field and thus a very large range of possible oscillations exists. The paper discussed some basic properties of these waves.

A fascinating combination of plasma and microwave physics was presented in a paper by G. S. Kino and B. Ludovici (Stanford). The paper discussed a plasma parametric amplifier based on the principle that if electromagnetic waves of three frequencies ω, ω_1 and ω_2 such that $\omega = \omega_1 + \omega_2$ can be propagated through a loss-less non-linear medium with propagation constants β, β_1, β_2, strong interactions will take place between these signals if $\beta \approx \beta_1 + \beta_2$; if ω is of large amplitude there will be a power transfer to ω_1 and ω_2. This principle has been confirmed in a mercury vapour d.c. discharge with $\omega = 800$ Mc./s., $\omega_1 = 500$ Mc./s., and $\omega_2 = 300$ Mc./s. Both ω_1 and ω_2 have been observed to increase in amplitude by a few db. in travelling from one end of the positive column to the other.

The broadening of spectral lines by Stark effects was discussed by H. Margenau (Yale). Equations for the calculation of half-widths were given for three different cases : (i) when both electrons and ions can be treated by impact theory, (ii) when the electrons can be treated by impact theory but the ions have to be considered statistically, and (iii) when both electrons and ions can be treated statistically. Physical conditions to which these equations are applicable were discussed. Improved methods of calculating Stark broadening of spectral lines were presented in a paper by H. R. Griem (Maryland) and A. C. Kolb (Washington). It was shown that the calculated line profiles depend only slightly on temperature and can therefore be used to deduce electron densities in dense plasmas from measured profiles with much improved accuracy.

Production, Confinement and Heating of Plasmas

This section, as with the other sections of the Conference, was split into sub-sections which were : (*a*) the longitudinal pinch ; (*b*) mirror machines and the azimuthal pinch ; (*c*) shock waves ; and (*d*) further methods of production and confinement.

In a paper by D. W. Allan (London), consideration was given to the detailed behaviour of the simple unstabilized pinch in respect of the inward movement of the current sheet. He concluded that evidence favours the free-particle piston model and the shock-wave model rather than the snow-plough model. Whether the behaviour follows more closely the free-particle model or the shock-wave model depends on the effective mean free path.

Two papers by S. A. Colgate, H. P. Furth and others (Livermore) discussed the linear and toroidal hard-core or inverse pinch. Small-scale instabilities in the linear case have been shown to be of non-hydromagnetic origin. A toroidal version of the hard-core pinch has been attempted using a magnetic field to levitate a ring conductor inside a toroidal shell. This device will be used to study the nature of these small-scale instabilities and also to study the stability of near-vacuum field hard-core configurations and to determine if this stability leads to an improved containment of the plasma energy. "A Dynamically Stable Current Column" was the title of a paper by V. S. Komelkov and others (Moscow). The formation and development of a current cord appearing during the movement of a plasma jet were examined by means of advanced high-speed photographic techniques. Measurements were made of the current distribution in the moving plasma jet and showed that the current in the cord had remained stable throughout the half-period of the discharge. The existence of a radiation continuum the appearance of which coincided with the appearance of the current cord had also been demonstrated.

A. Kantrowitz and others presented a paper on the use of collision-free shocks to study dissipation mechanisms in collision-free plasmas. At high temperatures, collisional dissipation in plasmas is slow, and losses due to magneto-hydrodynamic turbulence become important. Preliminary experiments show that it is possible to produce shock waves that obey the required theoretical condition that the shock thickness is less than the mean free path, and that observations on these thin shocks can provide a powerful tool for the study of dissipative mechanisms in collision-free plasmas.

Finally, L. Högberg, K. Siegbahn and K. Bockasten (Uppsala) described an apparatus for the electrodeless generation and acceleration of plasma rings. A single-turn primary winding placed close to the end wall of a 'Pyrex' tube induces a ring discharge which is accelerated as a function of the gas current and the magnetic field. Ion velocities in the range 10^6–10^7 cm./sec. have been observed with this technique.

Conclusion

During the Conference, visits to the Institute of Physics and the Institute of High Tension Research at Uppsala and the Nobel Institute of Physics in Stockholm were organized ; a full social programme for members and their wives was also arranged. All those concerned with the organization of the conference and particularly the hard-working secretary, Dr. Åke Nilsson, of the Institute of Physics, Uppsala,

are to be congratulated on the excellence of the arrangements which enabled such a large conference to run so smoothly throughout.

It is no reflexion on this organization to comment that these conferences, including as they do such a wide range of topics, are becoming too large and unwieldy; one possible solution would seem to be to divide the subject-matter into two groups, one devoted to ionization phenomena and gas discharges and the other to controlled thermonuclear research and plasmas. If these groups were arranged to run consecutively it would give those with interest in both fields an opportunity to attend more of the lectures in which they are interested, while considerably reducing the total number of delegates present at any given time.

The next conference in this series is to be held in Germany, probably at Baden-Baden, during 1961.

J. Dutton
D. Harcombe
E. Jones

[1] Garton, W. R. S., and Latham, R., *Nature*, **180**, 790 (1957).

RADIO ECHO OBSERVATIONS OF VENUS

By J. V. EVANS and G. N. TAYLOR
Jodrell Bank Experimental Station, University of Manchester

DURING September 1959 an attempt was made to observe radio echoes from the planet Venus using the 250-ft. radio telescope at Jodrell Bank. The radar equipment used with the telescope operated on a frequency of 408·00 Mc./s. The transmitted power was 50 kW., pulse-length 30 msec. and pulse repetition rate 1 per sec. The receiver had a noise figure of 4·6 db. and a bandwidth of 60 c.p.s. The overall losses in the feeders amounted to 2·5 db., and the polarization of the transmitted wave was right circular. Observations were made with the telescope in continuous motion to follow the planet across the sky, with alternate periods of transmitting and receiving. The length of these periods was approximately equal to the time of travel of the radio pulse to and from the planet (5–6½ min.).

No echoes were observed with this equipment stronger than the noise-level in the receiver. An analysis of the signals for echoes which were weaker than the noise was made with an integrating equipment, which added together receiver noise powers corresponding to the same range-intervals on successive sweeps of the time-base. With this system eight adjacent range-intervals were examined. These were made equal in width to the transmitter pulse (30 msec.) and their distance along the time-base was controlled in order to compensate for the change in range of Venus, so that any echo would remain in the same 30 msec. time-interval. It was also necessary to provide compensation for the Doppler shift of the echo relative to the transmitter frequency.

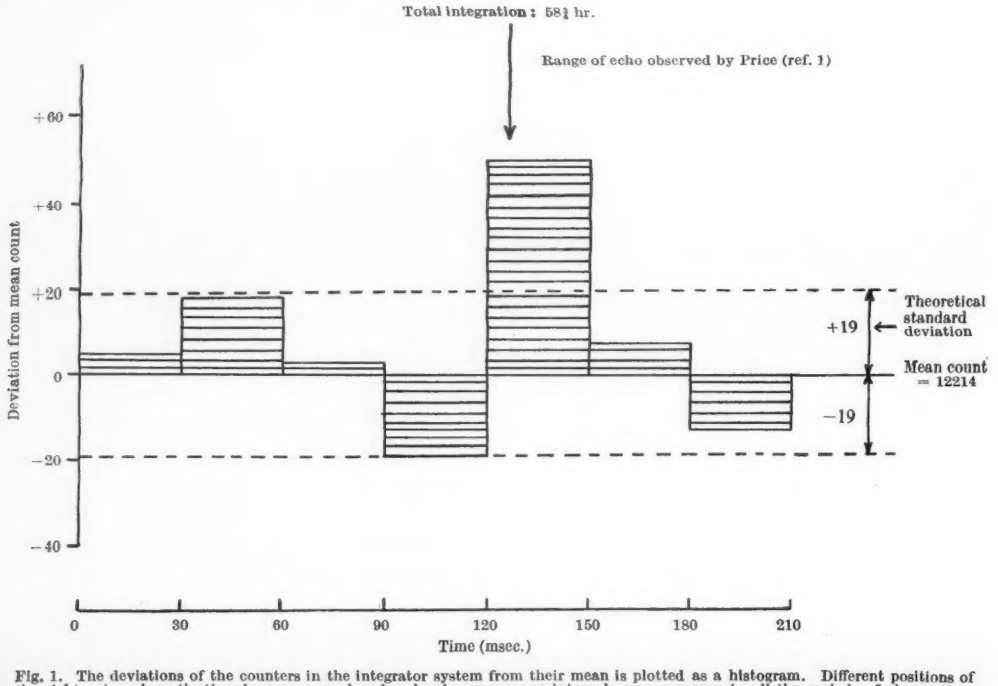

Fig. 1. The deviations of the counters in the integrator system from their mean is plotted as a histogram. Different positions of the eight gates along the time base were employed and only seven range-intervals were common to all the periods of observation. Hence there are only seven counts shown in the histogram

Exhaustive checks were made to confirm that the integrated noise counts in the eight range channels showed statistical fluctuations which agreed with theory, and that no systematic errors were introduced by, for example, the operation of the transmitter or the telescope. Such systematic errors were unlikely because the transmitter and receiver operated alternately for 5–6 min. intervals.

A total of 58¾ hr. useful operating time was obtained before the range of Venus had increased to a point where further work was not considered worth while. The addition of all the periods of observation is presented as a histogram (Fig. 1) where one of the eight range-intervals shows an excess count of 2½ times the standard deviation. The addition of many samples of noise by the integrating equipment gives a Gaussian distribution of counts, hence there is an 8 per cent chance that noise alone will produce a count 2½ standard deviations greater than the mean in one of eight channels. If the high count is caused by the planetary echo, then the range observed gives a value for the solar parallax of 8·8020 ± 0·0005 sec. of arc. This value is in agreement with that obtained by Price *et al.*[1] using the Millstone Hill Radar Station operated by Lincoln Laboratory of the Massachusetts Institute of Technology (8·8022 ± 0·0001 sec. of arc). The likelihood that, by coincidence, a high count should appear in the range interval predicted by the Millstone Hill result is 1 per cent.

The signal-to-noise ratio required to produce an excess count of 2½ standard deviations has been estimated by applying to the receiver signals which are weaker than the noise by a known amount, and is −23 ± 2 db. This is within 6 ± 2 db. of that expected on the basis of a model for Venus in which it was assumed that the scattering is similar to that observed for the Moon[2] and that the period of rotation is of the order of 20 days. However, the result is not in agreement with the work reported by Price, which suggested that the radar cross-section of Venus was equal to the physical area presented by its disk. If this were the case a signal-to-noise ratio of about −5 db. should have been observed. This discrepancy of 18 db. in signal strength might be accounted for in two ways: (*a*) if the rotation period of Venus is much faster than once every 20 days the Doppler broadening of the echo will cause some of the received power to fall outside the 60 c.p.s. bandwidth limit of the receiver; (*b*) if the echo is subject to large rapid changes in intensity then because a square law detection system was employed at Millstone Hill, a false estimate of the average signal-to-noise ratio would be obtained. Such fading could not have been caused by Faraday rotation in the Earth's ionosphere because circularly polarized radio waves were used in both experiments.

We are indebted to our colleagues at Millstone Hill for their interest and co-operation, particularly in producing for us predictions of the range and Doppler corrections which were applied to select the received signal.

[1] Price, R., Green, P. E., jun., Goblick, T. J., Kingston, R. H., Kraft, L. G., jun., Pettengill, G. H., Silver, R., and Smith, W. B., *Science*, **129**, 751 (1959).

[2] Evans, J. V., *Proc. Phys. Soc.*, B, **70**, 1105 (1957).

OBITUARIES

Prof. M. Caffrey

THROUGH the death of Prof. M. Caffrey, which occurred on September 17 at the age of seventy, Ireland has lost an outstanding personality in the field of agricultural science and one who took an active part in the Faculty of Agriculture in the National University of Ireland.

Michael Caffrey was born at Lughill, near Monasterevan, Co. Kildare. He received his early education at the local national school and at the Christian Brothers' School, Monasterevan. He entered the Albert Agricultural College, Glasnevin, in 1908, and in the following year was awarded an agricultural scholarship into the Royal College of Science, Dublin. After a distinguished undergraduate course, he gained the diploma of the latter institution in 1912, and was appointed as assistant to Dr. H. Hunter in the Plant Breeding Section of the Department of Agriculture, Dublin. The studies and work he then undertook under the able guidance of Dr. Hunter formed the basis of his later successful career in plant breeding, lecturing and teaching. When Dr. Hunter resigned after the First World War, Caffrey became head of the Plant Breeding Section of the Department of Agriculture, and when a Faculty of Agriculture was established in the National University of Ireland in 1927, the University made him lecturer in plant breeding, and afterwards in 1938 appointed him to fill the newly established chair of plant breeding, a post which he occupied until his death.

Throughout his career, Prof. Caffrey remained in close contact with the Department of Agriculture, which he kept supplied with nucleus stocks of leading cereals and grasses. These in turn became available to the various county committees of agriculture, by which they were tested and reported on in due course. Prof. Caffrey was also in close contact with plant breeding stations abroad, and he introduced many foreign cultivars of wheat, oats and barley. They were tested against cultivars commonly grown in Ireland and numerous crosses made with the most promising sorts. During four decades he produced not only varieties of wheat suitable to local soil and climatic conditions, but also improved varieties of oats and grasses. In crossing and breeding, Prof. Caffrey was particularly interested in the reaction of the hybrids to disease resistance, and one outstanding case of his work on this aspect may be quoted here. At Glasnevin, year after year, the most common and serious disease of wheat has been yellow rust (*Puccinia glumarum*). To combat this, Caffrey produced the cultivar Glasnevin Rosa, a wheat which was immune to yellow rust for seven years, a period which is about usual for varieties bred immune or resistant to rust diseases before they become attacked by new strains of the pathogen which develop in the meantime.

Close co-operation always existed between the Plant Breeding Division and the Plant Pathological Division at Glasnevin; and down the years the latter Division has been indebted to Prof. Caffrey on many occasions for directing attention to outbreaks of disease and to the appearance of new pathogens on cereal crops.

Although Prof. Caffrey's entire professional career was devoted to plant breeding, he had a wide interest in all agricultural subjects. He was a founder Council

member of the Irish Grassland Association; a member of the Agricultural Commission appointed by the Government to examine agricultural development in the late 'thirties; and in general he identified himself with many agricultural developments. He was equally at home with students and scientific audiences, and as a lecturer on behalf of the Royal Dublin Society he became intimately acquainted with and highly appreciated by a wide range of farmers all over the country.

Prof. Caffrey's outspoken manner, genial personality and hearty laugh will long be remembered by all who knew him. He was predeceased by his wife some years ago, and he is survived by three sons and three daughters, for whom the greatest sympathy is felt.

R. McKay

Dr. E. J. Holmyard

Eric John Holmyard was born on July 11, 1891, at Midsomer Norton, Somerset, and all his life he was a faithful man of Somerset. He was a scholar of Sidney Sussex College, Cambridge, and after graduation he served as a sixth-form science master at Marlborough College during 1917–19. He then became head of the Science Department at Clifton College, an appointment which he filled with signal success for the twenty years 1920–40. It was at Clifton that his best work was done. In 1941 he became editor of *Endeavour*, retiring in 1954 to live at Clevedon, Somerset. Among his other activities, he was chairman of the Society for the Study of Alchemy and Early Chemistry and co-editor of a "History of Technology" in five volumes.

Holmyard, as a teacher, well knew the capacities of young pupils at school, and his books on inorganic and organic chemistry of this standard have deservedly been very popular. They are written in a lucid and attractive style and many readers of this notice must owe their introduction to chemistry to them. In collaboration with F. A. Philbrick, he wrote a more advanced book on theoretical and inorganic chemistry which has also been very successful. All these books present the basic facts of chemistry as an experimental science, relating them to general principles in a way which gives them significance and interest; but the theory is kept in proper proportion, so that those who gained their knowledge from them in the past will now have very little to unlearn.

Dr. Holmyard, who was a member of the Royal Asiatic Society, will probably be best remembered for his profound studies of Muslim chemistry. He was well equipped with a knowledge of Arabic, and in this field he was a recognized authority. He made a special study of Jābir ibn Ḥayyān and the writings attributed to him. He published some Arabic texts, brought to light some little-known works of Jābir, and re-interpreted some which had previously been studied. More recent research has shown that the problem of Jābir is very difficult and much remains to be cleared up, but Holmyard's pioneering work has a permanent value. He showed that the theory which dominated alchemy and early chemistry, that metals are composed of mercury and sulphur, was taught by Jābir, who derived it from a statement in the "Meteorology" of Aristotle.

In collaboration with his pupil at Clifton, Mandeville, Holmyard published the Arabic text and a translation of a work known in Latin as by Avicenna and showed that it is, in fact, part of the genuine *Shifā'* of Ibn Sīnā. This text, which denies the transmutability of species and stigmatizes alchemical gold as fraudulent, was a puzzle in the Middle Ages, when it was thought to be part of the "Meteorology" of Aristotle. Holmyard also edited and translated an alchemical text of Abu'l Qāsim al 'Irāqī. In all these studies he made much use of the writings of Jildakī, available only in manuscripts, and established their value as a source of information on Muslim alchemy. The work in this field by Holmyard completely changed the outlook on Muslim chemistry which prevailed when he began.

Holmyard wrote some excellent small books on the history of chemistry, the best known being his "Makers of Chemistry" and his recently published "Alchemy". These are accurate and authoritative, and it is to be regretted that he did not write a general survey of Muslim chemistry which he was so well qualified to undertake.

Holmyard was modest and unassuming, ready to put his knowledge at the disposal of those who asked it, with a cool and critical outlook in scholarship, expressing himself concisely and avoiding polemics. His learning sat lightly upon him, and for all he cared it could remain unknown in circles incapable of understanding it. He was open and friendly and in whatever company he found himself his quiet charm and delicate sense of humour, wholly free from malice, endeared him. He was a member of Clevedon Golf Club. He died at Clevedon on October 13, and among those who valued his knowledge and friendship his death leaves a vacant place which it will be hard to fill.

J. R. Partington

NEWS and VIEWS

Royal Society: Award of Royal Medals

H.M. the Queen has been graciously pleased to approve recommendations made by the Council of the Royal Society for the award of the two Royal Medals for the current year as follows: to Prof. R. E. Peierls, professor of mathematical physics in the University of Birmingham, for his distinguished work on the theoretical foundations of high energy and nuclear physics; to Prof. P. B. Medawar, Jodrell professor of zoology and comparative anatomy at University College, University of London, for his distinguished contributions in the field of tissue transplantation immunity and acquired tolerance.

Geological Society of London: Foreign Members

The Geological Society of London has elected to foreign membership the following distinguished geologists: Academician V. V. Belousov, of the Academy of Sciences, Moscow, in recognition of his studies on sedimentation and geotectonics; Prof. J. A. Broggi, of Lima, Peru, for his work in the advancement of geological science in Peru and his contributions to Peruvian geology; Academician D. V. Nalivkin, of the Academy of Sciences, Moscow, in recognition of his contributions to the geology of the Soviet Union and especially of his part in the preparation of the recently published geological map

of that country; Academician N. S. Schatsky, of the Academy of Sciences, Moscow, for his work on tectonics, stratigraphy and economic geology; Dr. F. Prantl, vice-president of the National Museum, Prague, in recognition of his distinguished researches in palæography, stratigraphy and palæontology; Prof. Norman D. Newell, of the American Museum of Natural History, New York, for his contributions to invertebrate palæontology and his work on reef deposition.

British Broadcasting Corporation Science Unit

A SCIENCE UNIT has been established by the British Broadcasting Corporation with the object of providing a more extensive coverage of science in sound programmes. The senior member of the Unit is Dr. Archie Clow, who joined the B.B.C. in 1945 and has produced many science series and individual talks, notably the two weekly series "Science Survey" and "Who Knows ?", in which leading experts deal with all kinds of scientific developments in a non-technical way. The Unit is also responsible for "Science Review" and Third Programme science talks and discussions. Recently, Mr. David Edge joined Dr. Clow in the Talks Department. Both received their earlier education at Aberdeen, the former at Aberdeen Grammar School and the latter at Robert Gordon's College. Mr. Edge did research work in radio astronomy for three years after taking his degree in physics at Cambridge in 1955.

A third member of the Corporation staff who is contributing to the expansion of science broadcasts is Mr. C. L. Boltz, who is now attached to the News Division as science correspondent (*Nature*, **183**, 1231; 1959). He formerly worked for seven years in a similar capacity in the B.B.C.'s European Service. Succeeding Mr. Boltz in the European Service is Mr. Bryan Silcock, who was born in Liverpool in 1933 and has since 1957 been an assistant editor of *Nature*. He went to Dartington Hall School in Devon, and after National Service in the West Yorkshire Regiment and the Royal Artillery, to Jesus College, Cambridge, graduating with honours in natural science.

British Commonwealth Education Liaison Committee

IN a written answer in the House of Commons on November 12, the Minister of State for Commonwealth Relations, Mr. C. J. M. Alport, stated that in accordance with the recommendations of the Commonwealth Education Conference, representatives of all member countries in the Commonwealth met in London on October 27 under the chairmanship of Sir Henry Lintott. It was proposed to establish a Commonwealth Education Liaison Committee, comprising one representative of each member country and of Nigeria, and in addition the United Kingdom would appoint a member to represent the other Colonial territories. This Committee would follow up and record progress on the schemes of assistance agreed at the Oxford Conference and would also consider suggestions for the further improvement of Commonwealth co-operation in education, and, in particular, it would prepare material for submission to the next Commonwealth Education Conference, to be held in India in the winter of 1961–62, at the invitation of the Government of India. The chairman would be Sir Philip Morris, and under the general direction of the Liaison Committee there would be a Commonwealth Education Liaison Unit, consisting initially of a director (who would also be secretary to the Committee) and one administrative assistant. The Unit would supplement normal direct dealings between the countries of the Commonwealth on education, and would deal on request with inquiries from education authorities in Commonwealth countries and generally act as a reference centre. The cost of the Unit would be shared between member countries of the Commonwealth.

United States and Great Britain to exchange Data on Advanced Gas-cooled Reactors

THE United States Atomic Energy Commission and the United Kingdom Atomic Energy Authority have signed a five-year agreement to exchange technical information on advanced gas-cooled reactors. The exchange, effective as from November 16, will be carried out under the terms of the agreement between the two countries for co-operation in the civil uses of atomic energy, which has been in effect since 1955. Data will be exchanged on development, design, construction and operation, as well as on related research and development, of the advanced gas-cooled reactors being built at Windscale, England, and on the U.S. experimental reactor project of this type at the Oak Ridge (Tennessee) National Laboratory. Information exchanged under this agreement will be made available to British and American industry.

British Book Exhibition in Moscow

A LARGE exhibition of British books and periodicals sponsored by the British Council and the Soviet Ministry of Culture is opening in Moscow on November 21 for a fortnight. A similar exhibition of Russian books and periodicals will be shown at the Festival Hall in London next February. This will be the largest exhibition of British books and periodicals to have been shown in the U.S.S.R. at any time, so far as is known. Between three and four thousand books will be exhibited together with six hundred periodicals, a display of posters and large photographs. The exhibition will be shown in the main lecture hall of the Lenin Library, one of the largest libraries in the world. The books selected by the British Council have been provided free of charge by British publishers through the co-operation of the Publishers' Association. The main emphasis lies on science and technology, although there are important sections dealing with the arts and the humanities. Under the terms of the agreement made with the Soviet Ministry of Culture, there are no sections on religion, politics or economics. Two-thirds of the periodicals are on medical and scientific subjects. At the end of the exhibition all the British material will be handed over to the Soviet Ministry of Culture for use in Russian libraries and cultural institutions.

New Zealand Research on Weed Transportation

WEED species are sometimes inadvertently introduced into one country from another, but it is not always possible to establish the means by which this occurs. A. J. Healy, of the Botany Division, Department of Scientific and Industrial Research, Christchurch, has given some particular instances of introduction of foreign species into New Zealand (*New Zealand Journal of Agricultural Research*, **2**, No. 2; April 1959). The first example is a straw envelope from a whisky bottle picked up in a rubbish

heap on a North Canterbury farm. The envelope contained a flowering stem of bindweed, *Calystegia sepium*, portions of an inflorescence of tall oat grass, *Arrhenatherum elatius*, and a bent grass, *Agrostis* sp. Such containers when discarded are generally thrown on rubbish heaps and other sites suitable for weed establishment. A plate-glass container from an English source, examined in the Wellington district, contained wheaten straw in which was found portions of Californian thistle, *Cirsium arvense*, cleavers, *Galium aparine*, willow weed, *Polygonum*, fruiting material of *Beta* sp., nipplewort, *Lapsana communis*, and a hemp nettle, *Galeopsis* sp. This straw was being used locally both for compost heaps and in racing pigeon cages, which would further serve to disperse the weeds throughout the country. An examination of the trouser cuffs of a tourist returned from a trip through Spain, France, Switzerland and Italy revealed fruits of Gramineae and Compositae.

University News: Birmingham

The title of reader in mathematical physics has been conferred on Dr. J. G. Valatin, senior lecturer in mathematical physics. The following appointments have been made to lectureships: Dr. H. B. Griffiths (pure mathematics); Dr. J. K. Brown (chemistry); Dr. M. E. Davies (botany); Dr. Nancy Montgomery (botany); Brenda Manly (zoology); J. Cohen (zoology); C. R. Sladden (biology in the department of zoology); Dr. D. J. Blundell (geology); I. R. Smith (electrical engineering); K. B. Haley (engineering production); Dr. V. G. Jenson (chemical engineering); K. A. Redish (computing in the Department of Mathematical Physics); N. A. Dyson (physics); N. A. J. Rogers (chemistry).

The annual lecture of the Institute of Education is to be named "The Raymond Priestley Lecture" in recognition of the help which Sir Raymond Priestley gave to the Institute of Education when the Institute was established.

Preliminary plans have been approved for a building for highway and traffic engineering as an addition to the new Civil Engineering Building.

Oxford

The following research grants are announced: from European Research Associates, Brussels, £1,200 for one year from September 1, for research on acetylene chemistry being carried out in the Dyson Perrins Laboratory under the direction of Prof. E. R. H. Jones; from the Department of Scientific and Industrial Research a grant not exceeding £3,400 for the year ending July 31, 1960, for the maintenance of the 140 MeV. synchrotron for nuclear physics research in the Clarendon Laboratory; from the Colonial Medical Research Council a grant not exceeding £2,500 for a further two years ending August 31, 1961, for the study of the sensory neurohistological changes in skin infected with leprosy, being carried out in the Department of Human Anatomy under the direction of A. G. M. Weddell, reader in human anatomy; from the Smith, Kline and French Research Institute a grant not exceeding £1,500 for research on the electrophysiology and pharmacology of smooth muscle to be carried out in the Department of Pharmacology by E. Bülbring; from the United States Public Health Service a grant not exceeding 30,600 dollars for the year beginning September 1, 1959, for research in the Department of Biochemistry under the direction of Sir Hans Krebs.

Announcements

Prof. C. F. Carter, Stanley Jevons professor of political economy and Cobden lecturer in the University of Manchester, has been appointed a member of the Council for Scientific and Industrial Research, in succession to Prof. E. A. G. Robinson, who retires on completion of his period of service. Prof. Carter's interests are in the field of applied economics. He is the author of "Industry and Technical Progress" (with Prof. R. B. Williams), published in 1957, and "Investment in Innovation", 1958; he is chairman of the Science and Industry Committee of the Royal Society of Arts, the British Association for the Advancement of Science and the Nuffield Foundation.

The Metallurgical Society of the American Institute of Mining, Metallurgical, and Petroleum Engineers, in association with Interscience Publishers, Inc., has announced the publication of a new series of books entitled "Metallurgical Society Conferences", each volume being the proceedings of a technical conference sponsored by the Society through one of its technical committees. It is hoped by these means to provide for co-ordinated and comparatively rapid publication of scientific and engineering papers of interest to metallurgists. The first volume in the series, "Flat Rolled Products, Rolling and Treatment", can be ordered from Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, New York (price 3.75 dollars).

The Population Council Incorporated of New York is making grants totalling 89,348 dollars over three years to the National Institute for Research in Dairying. The award is in support of researches on cervical mucus, with particular reference to the control of fertility, to be carried out in the Physiology Department under the direction of Dr. S. J. Folley.

Industrial and Trade Fairs, Ltd., announce that a conference on New Engineering Materials and Design will be held in conjunction with the first Engineering Materials and Design Exhibition at Earls Court, London, during February 22–26, 1960. Among the subjects selected for discussion at the conference will be safety factors and the appearance in design. Further information can be obtained from the Secretary for the Conference on Engineering Materials and Design, Drury House, Russell Street, Drury Lane, London, W.C.2.

At the annual general meeting of the Photobiology Group, held at the National Physical Laboratory, Teddington, on November 6, Dr. D. Vince, of the Department of Horticulture, University of Reading, was elected honorary secretary in succession to Dr. E. M. F. Roe.

The sixth National Symposium on Reliability and Quality Control in Electronics will be held at the Statler Hilton Hotel, Washington, D.C., during January 11–13, 1960. Further information can be obtained from Mr. R. Brewer, The Research Laboratories, The General Electric Co., Ltd., Wembley, Middlesex.

Errata. In the communication entitled "Effect of Sorbitol on the Urinary Excretion of some B Vitamins in Man" in *Nature* of September 19, p. 911, the numerical values of the ordinates have been inverted; thus, reading from below upwards, the values for riboflavin should be 150, 200, 250 μgm.; those for thiamine, 10, 20, 30 μgm.; those for N-methylnicotinide, 2, 3 mgm.

ASSISTANCE FOR UNDER-DEVELOPED COUNTRIES

THE Queen's speech at the opening of Parliament on October 27 referred to the Government's urgent concern to improve conditions of life in the less-developed countries of the world and its intention to promote economic co-operation and support plans for financial and technical assistance. This was warmly welcomed in both Houses in the subsequent debates. Lord Stoneham asked for further information on this matter and, emphasizing its urgency, stressed the need to seek international agreement to stabilize basic commodity prices, the fall in which in 1958 had cost the under-developed countries 2,000 million dollars. Only urgent and increasing financial and technical assistance can avert the human suffering implicit in the two-thirds increase forecast in the population of Asia in the next fifteen years. The Marquess of Lansdowne was unable to specify the exact increases but assured Lord Stoneham that the Government intended to increase considerably contributions in the various fields of aid to under-developed countries. Lord Home also expressed the view that the foundation of peace probably lies in bringing the standard of living of the under-developed nations nearer to that of the industrial nations. In replying on the debate, the Lord Chancellor said that under the Colonial Development and Welfare Acts, £140 million would be available for the Colonial territories during the next five years, with up to £100 million more by way of Exchequer loans. External private investment of all kinds averaged £90 million a year, two-thirds being from the United Kingdom, and it was estimated that the United Kingdom's financial contribution to the Colonial territories averaged £100 million a year in 1956–58. Economic and technical assistance to all overseas countries and territories from United Kingdom public funds rose by a third in the past financial year to about £100 million, and is expected to increase similarly this year, exclusive of military assistance and certain emergency and miscellaneous expenditure amounting to about £30 million. Subject to agreement on the constitution for the new International Development Association, Parliament would be asked to put £50 million into the new Association.

In the House of Commons, Mr. J. Harvey, referring to the growing awareness of the need to give greater assistance to the under-developed territories, suggested that the Government might take some initiative in stimulating such interest so as to enlist voluntary contributions in addition to Government funds for this purpose. Mr. W. Owen suggested the Co-operative movement as a possible source of experience, knowledge and enterprise in this connexion, and Sir John Barlow advocated use of the International Monetary Fund to stabilize world production and prices of such primary commodities as tin and rubber. Mr. H. A. Price, pointing out that we are already devoting more than 1 per cent of our national expenditure to the under-developed countries, thought that we could do much more and that these territories offer great potentialities for the production of increased wealth. The President of the Board of Trade, Mr. R. Maudling, referred to our need to increase our balance of payments position if we are to play our full part in helping the development of these countries, and Mr. J. Arbuthnot suggested that reduced taxation in these countries would greatly assist in the creation of the conditions for development.

Mr. Anthony Head emphasized the vital importance of giving adequate attention to the backward, dependent and under-developed countries during the next five or ten years. He directed attention more particularly to the educational problem, and urged that here the West needs to overhaul its whole approach, and that co-ordinated effort is imperative. In this he was supported by Mr. K. Zilliacus, who pointed out that economic co-operation and the provision of financial, economic and technical assistance to backward countries internationally through the United Nations represent the support of constructive and modernizing forces. Sir Henry d'Avigdor-Goldsmid and Mr. J. Grimond spoke strongly in the same sense, Mr. Grimond referring also to the importance both of the type of government which is established in the newly independent territories and of education, including technical education. Mr. Philip Noel-Baker was somewhat critical of the magnitude of our present contribution. Although our contribution to United Nations Technical Assistance has risen from 2·5 to 3·0 million dollars, the Commonwealth has every year received far more from Technical Assistance than it has paid in, and against the increase in our contribution to the United Nations Special Fund from 1 to 5 million dollars should be set the schemes, costing 15 million dollars, for work in the Commonwealth already submitted by the Colonial Office to Mr. P. Hoffman. Moreover, the £1,000 million loan to the under-developed countries by the International Bank during the first twelve years of its existence was less than one-tenth of the extra capital required during the following ten years to achieve Mr. Hoffman's objectives.

Replying on the debate, the Minister of State for Foreign Affairs, Mr. J. Profumo, recognized the importance of the struggle for men's minds and the part which the Chancellor of the Duchy of Lancaster has to play in that connexion. He also took up the point about education, which had been stressed separately in the Queen's speech in a reference to the introduction of legislation to implement recommendations of the Commonwealth Education Conference, which had been warmly welcomed by Mr. E. Gardner and, in the House of Lords, by Lord Hastings. Lord Home, noting that the presence of 42,000 overseas students in Great Britain put a considerable strain on our universities and technical colleges, welcomed Lord Hastings's reference to the importance of education; nevertheless he thought that the task of equipping youth to meet the intellectual, physical and moral challenge of the time will strain our resources to the full. Apart from a reference by Mr. P. Wall on November 2 to the way in which the under-developed countries in Asia, Africa and the Middle East are beginning to realize the importance of European capital and European technicians, there was no further reference to the under-developed countries in the debate on the Address, although others besides Mr. Wall stressed the importance of education when the position in Central Africa was discussed at some length on November 2.

NEW RESEARCH LABORATORIES FOR THE CAMBRIDGE INSTRUMENT COMPANY, LTD.

By Dr. M. C. MARSH
Head of Research Department

ON October 14 a new block of research laboratories for the Cambridge Instrument Company, Ltd., was opened by Lord Adrian, Master of Trinity College and until recently Vice-Chancellor of the University of Cambridge. Following the opening, about 120 distinguished guests were entertained to lunch by the Directors of the Company, and they afterwards had an opportunity to inspect the new accommodation. On October 16 an open day was arranged for shareholders and for guests from the University and from several research establishments in the neighbourhood of Cambridge. About four hundred guests availed themselves of this opportunity. On both occasions the guests were received by Dr. P. Dunsheath, chairman of the Board of Directors, and Mr. H. C. Pritchard, managing director of the Company.

This new building is the result of a decision to expand greatly the research and development facilities of the Company. It provides about three times the previous floor area and permits the whole of these activities, which before this were dispersed in various parts of the works, to be brought under one roof, together with a design and drawing office.

The new laboratories are situated in Chesterton Road, adjacent to the Cambridge factory. They have a very fine view over the River Cam and over Jesus Green and provide accommodation suited to the work to be undertaken. As will be seen from Fig. 1, the clean, modern appearance of the building is emphasized by large windows that run the length of the first three floors and also by the colour contrast afforded by the light buff brickwork of the side walls and the dark green of the slate panels beneath the front windows. The building, which has a floor area of approximately 20,000 sq. ft., has four storeys and is provided with a three-storied entrance block and a rear link block giving access to the factory buildings. The mode of construction gives a clear area on each floor entirely uninterrupted by columns or beams, and extensive use has been made of modern building materials and fittings, such as red thermoplastic flooring, heating coils embedded in the structure, double-glazed windows and acoustic panels.

The entrance block is distinguished by a spacious entrance hall, which is decorated in contemporary style and contains an instrument showroom and a reception area. The block also houses offices and a lift serving all floors.

The laboratory block consists of four floors approximately 100 ft. long and 40 ft. wide. On the ground floor is the mechanical engineering laboratory with its offices, stores and constant-temperature room. Instrument makers' benches and experimental benches are arranged mainly beneath the front windows, and the remaining floor space is taken up by precision machine tools and mechanical laboratory equipment. The first floor is devoted to physics and electronics. It has a large and airy main laboratory, offices, dark room and optical laboratory, standards room and electronics workshop. The second floor houses the physical chemical laboratory which, in addition to offices, has special rooms for balances, glass-blowing and for chemical preparations. Polythene and 'Vulcathene' fittings are used in a special system for draining chemical waste from this floor. The top floor is taken up by design and drawing offices. Excellent lighting is provided by a number of roof-lights in addition to windows running the entire length of both walls. Along the front of this floor is a covered balcony, edged with flower-boxes.

In designing the laboratories, great stress has been laid on versatility. With this in view all the services are laid in trunks of ample size, and it would be an easy matter to add any facilities not originally provided. Besides the usual supplies of gas, water and electricity, there is a special low-voltage electrical supply for portable apparatus. At 18 places there are boards containing four terminals and an appropriate switch. From a central control board it is possible to feed to these terminals a three-phase and neutral supply of any required voltage, single-phase a.c. of any voltage up to 250 volts at frequencies between 25 and 60 c./s., a.c. stabilized against changes of voltage and frequency and d.c. between 0 and 250 volts. These supplies are obtained from trans-

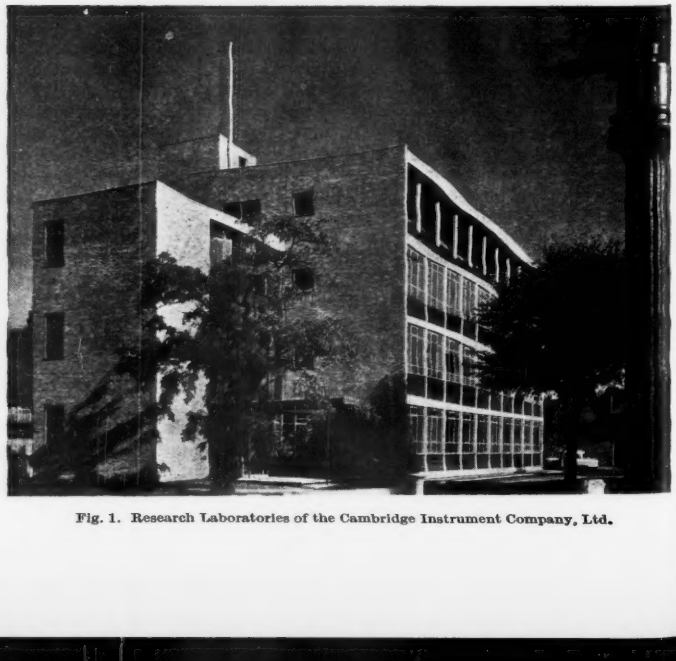

Fig. 1. Research Laboratories of the Cambridge Instrument Company, Ltd.

formers, stabilizer and a Ward Leonard set with a connected motor alternator. The last two are situated in a cellar but are controlled from two points in the laboratories. On the third floor a large glass de-aerator of special design has been fitted in order to supply oxygen-free water for researches on boiler feed-water problems. Compressed air of high and low pressure is supplied to all laboratories, with an extra high-pressure supply in the mechanical laboratory.

The building was completed in the remarkably short time of just under one year by Messrs. J. Jarvis and Sons, Ltd. (London and Manchester), to the design of the architects, Messrs. Edward D. Mills and Partners. Light oak furniture with teak tops has been supplied to all the laboratories by Messrs. Baird and Tatlock (London), Ltd.

These laboratories are now fully occupied and, with an expanding staff, a large programme of research and development work is being undertaken. This programme relates to improvements to instruments which are already being manufactured, as well as to a number of entirely new projects in which the Company is interested.

CARBON-DATING CONFERENCE AT GRONINGEN

SEPTEMBER 14–19, 1959

ON the invitation of Prof. Hl. de Vries (Natuurkundig Laboratorium) and Prof. H. T. Waterbolk (Archäologische-Biologisches Instituut), of the University of Groningen, a small conference of scientists from carbon-dating laboratories was held in Groningen during the week September 14–19. It was similar in character to the conferences held in Copenhagen, Cambridge and Andover, Mass., already reported in *Nature*[1,2] and *Science*[3]. Twenty-two dating laboratories were represented, some well established and others just getting into their stride, from twelve countries. We were happy for the first time to welcome Russian colleagues at these meetings. Some thirty-five communications were made to the meeting, which was partly concerned with the technique of carbon-dating and partly with selected aspects of its application to geological and archæological problems.

There was less emphasis than hitherto upon the techniques of counter and circuit design, but nonetheless there was a very interesting survey of the methods in use at various laboratories. Two laboratories described their scintillation counting techniques, that at Saclay, using paraldehyde, and that at Trinity College, Dublin, using methanol. While a few laboratories use acetylene or methane in their proportional gas-counters, it seems that the majority of dating laboratories now favour carbon dioxide counting. Considerable emphasis was laid on the need for careful pre-treatment of samples, especially with difficult materials such as bone, charcoal, cave-earths and all samples of great age. Accounts were given of several promising investigations into matters affecting the principles of the method and its applications, as, for example, those at Heidelberg illustrating possible seasonal variations of radiocarbon content of the atmosphere. Particular interest was attracted by an account of the joint investigations of Cambridge, Copenhagen and Heidelberg upon possible fluctuations in the initial atmospheric carbon-14 concentration during the past 1,200 years; this is a promising extension of ideas recently suggested by de Vries[4] as possibly offering insight into past climatic changes.

Without attempting to summarize all the interesting contributions, it may suffice to point to two or three fields in which the application of carbon-dating has now apparently led to a highly significant advance in knowledge. First, we may note a series of datings made at Groningen of the earliest Neolithic cultures from south-eastern and central Europe, indicating a spread from the Near East across these regions as early as 4000 B.C.; these findings were paralleled to some extent by numerous datings made in Pisa upon Italian material.

Several contributions concerned the dating of stages of the last glaciation characterized by stratigraphy, archæology or biological and climatic evidence. Here the contribution of Groningen was particularly important, since the technique of isotopic enrichment had permitted the addition of several half-lives to the maximum possible age attainable[5]. With favourable materials that warrant this costly and lengthy process, ages as great as 64,000 ± 1,100 years are attainable. This in fact appears to be the date of the first mild interstadial period after the last (Eemian) interglacial. There is considerable evidence now for an interstadial about 30,000 years ago at several places in western Europe. From the American laboratories comes very convincing evidence obtained by dating ocean cores, and deposits both in the Caribbean and in salt lakes, for a very abrupt amelioration of climate about 11,000 years ago. It is striking that this climatic break corresponds exactly with the well-dated Late-Glacial period of climatic change in Europe.

A substantial part of the time of the meeting was properly devoted to various matters of co-ordination of the work of different laboratories. The successful outcome was reported of two policy decisions taken at earlier conferences, namely, to establish an agreed system of publication of date-lists and to publish a punch-card system of all published dates. The first of these objectives has been met by the publication of the Radiocarbon Supplement of the *American Journal of Science*, the first volume of which appeared in May of this year. The second was met by the formation of Radiocarbon Dates Association, Inc. Mr. Fred Johnson gave a description of the principles he had adopted in designing the punch-card system and reported the first issue of cards to subscribers. It is not yet sufficiently widely known by archæological, geological and biological laboratories concerned with the history of the past 70,000 years how massive is the contribution already made by carbon-dating to knowledge of this period, nor what a very rich source of information this punch-card system will provide. (Inquiries for subscriptions to the carbon-dating punch-card index should be directed to Radiocarbon Dates Association, Inc., Robert S. Peabody Founda-

tion for Archæology, Philips Academy, Andover, Massachusetts, U.S.A.)

With regard to future procedure, it was decided that all carbon-dating laboratories should check by a common standard and that this should be the oxalic acid standard of the U.S. Bureau of Standards. It was agreed that a value of 95 per cent of this standard activity could be taken as the agreed radiocarbon activity for organic material (but not shells) originating in A.D. 1950. This decision should remove many of the minor difficulties caused by the different laboratories having individual standards of contemporary activity on which to base their calculations of age, and it is hoped that either the next or the next but one date-list of every laboratory will be based upon this agreed standard, which will take care of the industrial carbon and hydrogen-bomb effects upon recent samples. It was at the same time recognized that the carbonate sample provided by Heidelberg would be a further check of importance; that laboratory has undertaken the co-ordination of all inter-laboratory calibration measurements.

It was agreed to defer decision on a carbon-13 standard, pending exact absolute determinations to be made in the Lamont Laboratories.

It was agreed to use the methods of presentation of bibliography now employed in the Radiocarbon Supplement and in *Quaternaria*: the conference also recommended that new dating stations should adopt as their index letters the most distinct and simple combination possible, avoiding those that have already been used even by stations not at present producing dates.

While there was no agreement as to whether dates ought preferably to be expressed as B.P. (before the present), or B.C. (and A.D.), there was considerable sympathy for the view that dates primarily relevant to archæology should be given in the B.C./A.D. scale, even where the date B.P. had also been given.

Members of the conference had the concentration of lecture room attendance broken by visits to Prof. de Vries's laboratory, to the great peat bog and moraine region south of Groningen and to the dramatic areas of reclamation where carbon-dating is assisting the Geological Survey to provide fundamental knowledge of the stratigraphy of coastal deposits.

The thanks of all participants are due to all our Dutch hosts, to the Rektor Magnificus of the University of Groningen, to the Royal Dutch Shell Company, to the Chief Engineer and Director of the Rijks Waterstaat, and above all to the primary organizers of the conference.

H. Godwin

[1] Godwin, H., *Nature*, **174**, 868 (1954).
[2] Levi, H., *Nature*, **176**, 727 (1955).
[3] Johnson, F., Arnold, J. R., and Flint, R. F., *Science*, **125**, 240 (1957).
[4] Vries, Hl. de, *Proc. Kon. Ned. Akad. van Wetenschappen, B*, **61** (2), 1 (1958).
[5] Vries, Hl. de, Vries, A. E. de, and Harris, A., *Science*, **128**, 472 (1958).

ENZYMES IN THE FOOD INDUSTRY

THE Committee of the Food Group of the Society of Chemical Industry has an established reputation for organizing symposia on subjects of vital importance and with a wide range of interest to food scientists and technologists. The most recent, held on October 1–2, dealing with enzymes associated with the manufacture, storage and distribution of food, attracted an audience which taxed the capacity of the hall of the Royal Society of Medicine in which it was held. The organizers very wisely decided to limit the scope of the contributions and to divide them roughly into two groups: one dealing with the production of enzymes and their use in manufactured foods and the other with the activity, both useful and deleterious, of naturally occurring enzymes in foods.

Dr. Malcolm Dixon opened the symposium with a paper, giving in his own characteristic way the necessary background information on the types of reactions which may be catalysed by enzymes, and such of their properties as would have a bearing on the matters discussed by later speakers. The value of such an introduction to a symposium covering a broad field of biochemistry cannot be too highly stressed when it is appreciated that the audience was composed mainly of persons connected with the food manufacturing and processing industries, specialists maybe in rather limited fields, who frequently find it hard to keep abreast of fundamental developments.

The remainder of the first day was given over to papers dealing with fungal amylase, invertase, rennin, glucose-oxidase, the pectin-degrading enzymes and proteinases from plants and micro-organisms. The main interest in fungal amylase and invertase was in the methods adopted to secure conditions of culture of the selected organism so that high yields of high-purity enzyme are possible on a commercial scale. The discussion on the papers not unexpectedly centred around the newer applications of enzymes by the food industry and in particular the use of amylase in bread-making, the application of such proteinases as papain, bromelin (from pineapple) and ficin (from figs) to meat with the view of increasing its tenderness, and the recent availability of glucose oxidase as an oxygen scavenger in packaged foods.

The proceedings of the first day having presented the enzymes in a favourable light, as processing aids in a variety of food products, the second day, devoted to "Innate Enzymes: Their Action and Control", revealed the reverse side of the medal and showed enzymes in a less co-operative mood. This was not unexpected: Dr. Dixon, in his introductory remarks, had already pointed out that foods are the product of enzyme action in the living plant or animal and are metabolized after consumption by enzyme action in the body of the consumer, stressing the fact that in the living cell the urge of the enzyme processes is towards synthesis and that enzyme changes in foods, which can be regarded as post-mortem changes, may well be deteriorative in character. It is, however, sometimes difficult to draw the line; the enzymic ripening of fruit leads progressively into the deteriorative changes of over-ripening. Other cases are more specific: papers presented during the day dealt with enzymic deterioration in colour (blackening of potatoes by polyphenolase), in flavour ('soapiness' in coconut and palm kernel oil products due to liberation of free fatty acids by lipase action) and in nutritive value (oxidation of ascorbic acid and of vitamin A precursors in plant tissues).

The problem of control is a formidable one. Cold storage merely delays but does not prevent enzymic deterioration, since enzyme reactions, like all other chemical reactions, are slowed but not stopped by lowering of temperature. Other standard methods, such as the addition of inhibitors or competitive substrates, are by no means universally applicable. Probably the most commonly used procedure is that of heat inactivation ('blanching'), but this can lead to unwanted structural changes in fruits and vegetables. At present there is no simple and universal solution.

It must be said that on one point the arrangement of the symposium was open to critical attack, namely, the lack of time available for free discussion. It is in this discussion that the value of a symposium such as this largely resides, both for the audience and for the contributors themselves. Arrangements had been made for the discussions to be opened by appropriate authorities and these authorities presented what virtually amounted to additional contributions, comparable in weight and importance to those of the main contributors. On this account, the unscripted discussion was seriously restricted, particularly at the final session, and a number of potential questions had to remain unasked and unanswered. This was undoubtedly a loss and one can but ask that the point should be borne in mind when future symposia are being planned.

It is, however, pleasant to record that the symposium went with a swing to the end and was closed by the clock : there was no noticeable whittling away of the audience as special interests were disposed of. In its lighter moments the meeting considered future possibilities ; these included 'tailored' enzymes for specified purposes and the application of enzymes to the restoration of flavour in over-cooked cabbage. It was also pointed out that one speaker had coined a new word—the verb "to enzyme". The purists may refuse to accept this innovation, but we must all accept the importance of the process it describes.

BIOCHEMICAL RESEARCH IN INDIA

GOLDEN JUBILEE SYMPOSIUM

A SERIES of symposia was organized by the Department of Biochemistry, Fermentation and Pharmacology Laboratories of the Indian Institute of Science, as part of the celebrations of the golden jubilee of the Institute, during August 28–30, and was attended by more than two hundred scientists, including fifty delegates representing important centres of biochemical research in the country. The subjects covered were "Biology and Biochemistry of Micro-organisms", "Enzymes" and "Vitamins".

The symposia were inaugurated by Dr. S. Bhagavantam, the director of the Indian Institute of Science, and the first day of the session was presided over by Major-General S. L. Bhatia, who spoke on the "Progress of Physiology and Biochemistry in India". Prof. P. S. Sarma, who presided over the proceedings of the second day, outlined the contributions in enzyme chemistry made by the late Prof. K. V. Giri (see *Nature*, **182**, 1201 ; 1958). Dr. V. N. Patwardhan, who took the chair on the third and final day of the symposium, gave an address on the mode of action of vitamin D on which he and his group have been working for the past two decades. He and his collaborators have adduced experimental evidence to show that vitamin D acts presumably by promoting the synthesis of citric acid in the epiphyseal cartilage. Dr. V. Subrahmanyan, director of the Central Food Technological Research Institute, Mysore, who was professor of biochemistry in the Indian Institute of Science from 1931 until 1949, reviewed the work done in the department during his regime. He gave a brief account of the development of the Bangalore process of composting, the elucidation of the principles of sewage purification, the preparation of a material from paddy husk for defluorinating fluoride-containing waters and the preparation of insulin and other hormones from slaughter-house material and vegetable 'milk' from soya bean. Prof. M. Sreenivasaya, who was one of the pioneers in enzyme chemistry during the early years of the Biochemistry Department, described the elegant method developed by him for the study of enzymes both by the ultra-micro- as well as by micro-dilatometric methods.

Sixty-nine original research papers were presented at the symposia, and only a selection can be mentioned here.

M. G. Bhat, of the Indian Institute of Science, read a paper on the nutrition and metabolism of *Pseudomonas convexa* var. *hippuricum* representing the work done by her in collaboration with Drs. T. Ramakrishnan and J. V. Bhat. Detailed investigations with regard to the nutritional requirements and metabolic pathways of this organism, which was isolated from soil using the enrichment culture technique, were outlined and a new pathway of benzoate breakdown by the bacteria involving salicylate, a mechanism different from the classical scheme of the metabolism of the aromatic ring, described. M. K. Subramanyam (Indian Institute of Science) gave a résumé of his studies on the cytology of yeast, which included the demonstration of the presence of a nucleus and a vacuole as well as the occurrence of nuclear and vacuolar membranes in the yeast cell. He also pointed out the general similarity of the structures of yeast and plant nuclei.

M. Chakravorty and D. P. Burma, of the Bose Institute, Calcutta, presented a paper on "Microbial Synthesis of Protein in Relation to the Biogenesis of Nucleic Acids". Using phosphorus-32 and sulphur-35, they have shown that, in the resting cell of *Azotobacter vinelandii*, conditions under which nucleic acid synthesis is inhibited lead to a decrease in protein synthesis. On the contrary, it was found that the incorporation of phosphorus-32 into the nucleic acid continued in an uninterrupted manner even when protein synthesis was inhibited. P. S. Sarma and co-workers, of the University Biochemical Laboratory, Madras, working on metal requirements of nicotinamide deaminases, have investigated the inhibition by metal-chelating agents of nicotinamide-deamidating systems in cell-free extracts of micro-organisms and the soluble fractions of pigeon liver. A study of the reversal of the inhibition, produced

by α-α′-dipyridyl with various metal ions, has shown that the enzyme systems in the insect *Corcyra cephalonica* St., pigeon liver and chick kidneys are reactivated by Fe^{++}, that in *A. niger* by Mg^{++} and the one in *N. crassa* by Mn^{++}.

The detection and purification of a stereo-specific dihydrolipoic acid dehydrogenase formed the subject-matter of an interesting paper by D. K. Basu and D. P. Burma, of the Bose Institute, Calcutta. The enzyme, which was purified 60–70-fold, was found to be diphosphopyridine nucleotide-linked and specific for dihydrolipoic acid and its amide. The reaction was irreversible when tested with lipoic acid as the substrate. I. S. Bhatia and co-workers, of the Tocklai Experimental Station, Cinnemara, Assam, gave an account of their work on the transglycosidase present in tea leaves. This enzyme reacted with maltose with the formation of maltotriose, maltotetrose and glucose. With arabinose as the acceptor of glucosyl residues and maltose as the donor, a disaccharide containing glucose and arabinose was formed.

The purification and properties of glutamic–oxalacetic transaminase from ox brain and from human brain were described by T. N. Pattabhiraman and B. K. Bachhawat, of the Christian Medical College, Vellore. A 30–40-fold purification of the enzyme was achieved by fractionating the initial extract with alcohol, Zn^{++} and ammonium sulphate. The purified ox-brain transaminase showed complete dependence on pyridoxal phosphate for its activity.

A new type of enzymatic transamination reaction in which glyoxylate transaminates with a number of amino-acids to produce glycine was reported by L. V. S. Sastry and T. Ramakrishnan (Indian Institute of Science). *Iso*nicotinic acid hydrazide and L-penicillamine at low concentrations inhibited the enzyme but the inhibition was reversed by pyridoxal phosphate or metal. The authors adduced unequivocal evidence to show that the transaminase was a metallo-enzyme. The purification and properties of a naturally occurring inhibitor of glutamine synthesis present in *Pongamia* galls was described by N. K. Sukanya and C. S. Vaidyanathan (Indian Institute of Science); they also showed the preponderance of this inhibitor in the gall tissue, as compared to normal tissue.

N. Appaji Rao, H. R. Cama and S. A. Kumar (Indian Institute of Science) gave details of some of their recent work on the occurrence of flavin nucleotides in plants and the changes in their concentration with germination of green gram (*Phaseolus radiatus*) and cow pea (*Vigna catiang*). The radicle of the germinating seedlings contained almost all the flavin adenine dinucleotide and the major portion of the total flavin, while the cotyledons and the plumules contained flavin mononucleotide as the major flavin. Some interesting examples of species specificity in the mechanism of pyridine nucleotide synthesis by erythrocytes were reported by P. G. Tulpule, of the Nutrition Research Laboratories, Hyderabad. Of the seven species studied by them only human and guinea pig erythrocytes were capable of synthesizing diphosphopyridine nucleotide from nicotinamide and glucose. Human as well as monkey erythrocytes could also synthesize appreciable amounts of diphosphopyridine nucleotide from nicotinic acid and glucose in the presence of glutamine, whereas this metabolic pathway did not seem to operate in the guinea pig. Red blood cells of the monkey were able to synthesize diphosphopyridine nucleotide only in the presence of glutamine, suggesting that nicotinamide was converted to nicotinic acid prior to incorporation in diphosphopyridine nucleotide.

Four special lectures were given in the evenings on each day of the symposium: Dr. D. P. Burma, of the Bose Institute, Calcutta, on "Pentose Phosphate Metabolism", Dr. B. K. Bachhawat (Christian Medical College) on "Purification of Enzymes"; Dr. P. M. Bhargava (Regional Research Laboratories, Hyderabad) on "Protein Synthesis" and Dr. T. Ramasarma (Indian Institute of Science) on "Coenzyme Q".

P. S. Sarma

NATIONAL VEGETABLE RESEARCH STATION

NEW LABORATORY BUILDING

THE new laboratory building of the National Vegetable Research Station was officially opened on October 23 by H.R.H. The Duke of Edinburgh.

The decision to establish the Station was taken after the Second World War because of a continuing need to encourage vegetable production in Great Britain, and the Agricultural Research Council is now responsible for government grant-aid to finance the Station and for the general supervision of its scientific programme.

The new building marks the culmination of ten years development from the time when the director, Dr. J. Philp, took over 280 acres of land at Wellesbourne, near Warwick, in September 1949. Initially the site had no electricity, roads or suitable water supplies, and the only buildings were three small cottages and a few farm buildings. Building restrictions in the early years severely hampered development and the research staff had to be housed temporarily in old service huts, while Dutch light structures served as temporary glasshouses.

The new laboratory building was designed by Mr. F. W. Holder, chief architect of the Ministry of Agriculture, Fisheries and Food, and has a total floor area of 33,800 sq. ft. Besides laboratories and offices it contains a library, lecture room and committee room, and the basement has space for the future provision of controlled-environment chambers. The construction uses pre-cast concrete frame and floors with external curtain-walling of insulated plastic and some brickwork, the roof being of copper. The Station also has about a half-acre of glasshouses used for pot-experiments in research projects, a packing shed, implement shed, farm stores and buildings for livestock. 170 acres are served by underground irrigation mains with a borehole for the water source.

The site at Wellesbourne is central for the country as a whole and the soil and climate are suitable for vegetable production, being similar to those in the nearby Vale of Evesham, an important horticultural area. The area of 280 acres, to which 95 acres have

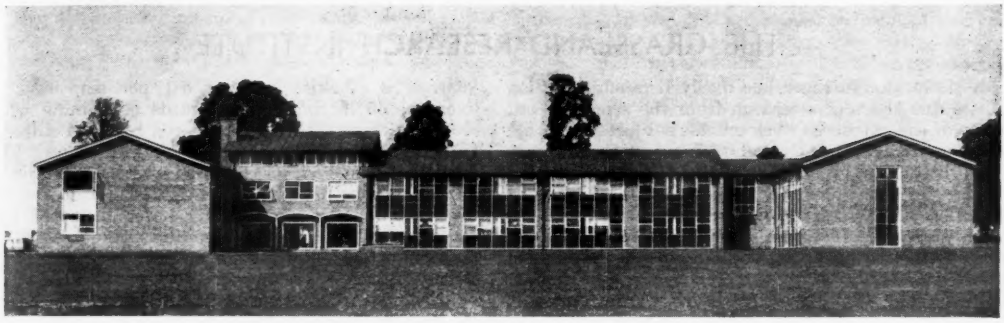

Fig. 1. New laboratories of the National Vegetable Research Station

recently been added, allows a farm unit to be run in conjunction with the experimental vegetable crops and is large enough to provide the isolation necessary for some of the research work. In addition, there is a sub-station at Paglesham, Essex, with an area of 150 acres, which is chiefly used for the multiplication of seed stocks.

All vegetable crops grown in the open and in cold frames, except potatoes, come within the purview of the Station, and its work is organized under eight sections: plant breeding; chemistry; physiology; irrigation; entomology; pathology; weed control; statistics.

Long-term experiments with a rotation of vegetable crops are now in their sixth year. The treatments comprise organic and inorganic fertilizers, time and frequency of application of nitrogen, and methods of soil cultivation. Growth studies are being made on some of the crops in these experiments to obtain information on the stages of growth most affected by fertilizers; the weed populations and soil-moisture characteristics of the soils under different cultivation methods are also being investigated. Dung has been shown to exert a marked influence on growth during the very small seedling stage.

Plant-breeding is carried on at a practical level with the production of improved varieties of vegetables, and at a more fundamental level with the application and development in vegetable crops of breeding methods as such. The method of inbreeding to attain uniformity followed by crossing to restore vigour, used so successfully in maize, has been applied to brussels sprouts, and hybrids with yields 40 per cent above the parent strains have been produced. The backcross technique is being used for the introduction of winter hardiness into an otherwise satisfactory quick-freeze variety of pea. In the dioecious species, asparagus, where the male plant is commercially desirable and usually heterogametic, isolation of homogametic male plants has produced strains giving all-male progeny on crossing.

Entomological research is mainly concentrated on the carrot fly, the cabbage-root fly and the lettuce-root aphid. Ecological work on the effect of insecticides on the balance between the cabbage-root fly and its predators has shown that the balance can be tipped in favour of the pest by indiscriminate broadcast application of insecticides. The use of insecticides against carrot fly raises problems of off-flavours in the crop and of the possible build-up of insecticides in the soil. Extensive testing tests have been carried out at the Station with carrots to assess the effect of insecticides on their flavour. A technique which has been developed for the assay of insecticide residues in the soil enables concentrations of dieldrin as low as 0·05 p.p.m. of soil to be measured.

The diseases of vegetable crops are as diverse as the crops themselves. The silvering disease of red beet, which is bacterial, has been successfully controlled with streptomycin. Fungal diseases include the long-known club root of brassicas and the relatively new crook-root disease of watercress; new fungicidal techniques give hope of controlling both these, while the prevention of virus diseases in watercress and lettuce is being sought through the production of clean stocks. The development of resistant varieties by selection and breeding techniques is being tried for parsnip canker and *Didymella* stem rot of tomatoes.

Work on the irrigation of vegetables has been primarily concerned with the most efficient use of water. Moisture-sensitive periods during growth have been found to exist with some vegetable crops, and at these periods irrigation has its maximum effect, while at others it does not produce an economic response. Work has been started on the relationship between water and nutrient uptake in the root system and on the use of additives to irrigation water.

The newer residual herbicides are being tested for suitability on a wide range of vegetable crops, and work has recently started on the nature and extent of the damage to vegetable crops caused by the drift of spray from herbicides used on neighbouring agricultural crops. The effects of weeds are also being studied in two other aspects: the unevenness of weed distribution has been shown to be partly responsible for field-plot variation in the yields of experimental crops, and the reduction in crop yields caused by weeds has been found to be affected by the spatial arrangement of the crop plants. Suitable mathematical models to describe the growth of some crop plants are being sought, with the view of improving the interpretation of treatment-year interactions in long-term experiments.

On the practical side, the Station maintains close links with the National Agricultural Advisory Service, and is fortunate in being able to make use of the experimental horticulture stations of this body for the further testing of experimental findings under a wider range of conditions. On the academic side, an arrangement with the University of Birmingham enables postgraduate work carried out at the Station to be recognized for the purpose of obtaining internal higher degrees of the University.

J. A. NELDER

THE GRASSLAND RESEARCH INSTITUTE

IN the past, grassland has received much less than its due share of attention from the scientist, but some of the extensive work on this subject now being done in Great Britain is summarized in the recently published annual report for 1957–58 of the Grassland Research Institute (Experiments in Progress, No. 11. Pp. 108. Hurley, near Maidenhead: Grassland Research Institute, 1959. 7*s*. 6*d*.). Many aspects of the composition, treatment and use of grassland swards are investigated by the nine departments. The Department of Herbage Agronomy is concerned with management, yield and quality of herbage at all seasons of the year, and the emphasis has shifted from comparison of species and varieties to establishment and management of the sward. Continuation of the study of the relationship between white clover and top-dressings of nitrogenous fertilizer has shown that the effect of white clover on gross yield of herbage was equivalent to approximately 9 cwt. per acre of a nitrogenous fertilizer annually on a no-clover sward. The results of experiments with gibberellic acid showed that response to nitrogenous fertilizer at 4 cwt. per acre was greater than response to 2 oz. per acre gibberellic acid. Nitrogen plus gibberellic acid produced an additive effect at first, but in the presence of nitrogen there was a significant depression in yield as a result of gibberellic acid treatment; depression was greatest where the initial response to gibberellic acid was marked.

In the Section of Animal Agronomy, grassland production is measured in terms of the animal. Better live-weight performance per animal was obtained when cocksfoot was grown with lucerne than when lucerne was grown alone. A comparison of two methods of sowing and managing lucerne and cocksfoot indicates that it is possible to extend the grazing season by about four weeks in the autumn by use of nitrogenous fertilizers. The live-weight gain per acre of ewe lambs carried throughout the year at a stocking rate of 6·1 per acre was, on average, 65 lb. higher on swards containing white clover, although the grass swards received nitrogen to compensate. The comparative influence of leys, variously managed, on the yield of subsequent cereal and kale crops has been investigated by the Department of Ley Agronomy. It is demonstrated that management of ley swards has an appreciable effect on yield of the following crops. This is accounted for largely by the nitrogen status of the soil. The study of the intake and digestibility of herbage is one of the main concerns of the Department of Biochemistry and Animal Nutrition. This involves both feeding experiments and biochemical studies of herbage plants. The former have shown that certain of the major grasses are more digestible than others and the latter that the older methods of fractionation are too arbitrary; modern techniques are likely to give a more reliable assessment of digestibility.

The work of the Department of Plant Physiology is linked with that of Herbage Agronomy in laboratory and field. The detailed growth studies are likely to provide valuable guidance on grazing practice. Experiments in microbiology are connected to soil and herbage studies in other departments, with emphasis on the examination of the processes of decomposition which take place when the ley is ploughed. A small experiment on sterilization of grass by radiation suggests that it may be possible to preserve grass for several months without undue changes in palatability. Extra-mural experiments provide supplementary evidence over a wide variety of environmental conditions. Such experiments are usually done in collaboration with the National Agricultural Advisory Service. The Department of Biometrics provides statistical advice and a computing service for other departments and has commenced its own field-experiments to answer specific questions.

SCIENTIFIC RESEARCH IN ALBERTA

THE thirty-ninth annual report of the Research Council of Alberta, covering the year 1958, stresses the work on ground water geology and the studies on the Precambrian Shield area of north-east Alberta (Report No. 78. Pp. 66. Edmonton: Research Council of Alberta, 1959). Work was commenced on the delineation of areas with large coal reserves which could be developed by industry as a source of power, and reconnaissance surveys were made of alkali lakes in Alberta and of the mountains west of Nordegg. A laboratory study of till from the Cooking Lake moraine showed that electrical potentials up to 0·5 V. can be generated in soil between individual horizons by natural processes. Soil surveys continued and a study is being made to determine the characteristics of the dominant parent materials of Alberta soils, the composition of the glacial till and the local variations. Further work at Youngstown showed that the productivity of the solonetz soils was limited by the physical condition of the soil, times and timeliness of irrigation being both critical under such soil conditions. The hail reporting network was further extended and valuable results were obtained in spite of an unusually low incidence of hail in the area. The highway research programme was largely concerned with the instrumentation and study of a five-mile portion of the trans-Canada highway west of Calgary, on which test sections of three different types of concrete pavement were laid.

Fundamental studies on coal in the organic chemistry laboratory included an examination of reactions of humic acids, a preliminary survey of the properties of kerogens and other organic substances associated with inorganic sediments, and substantial progress in the separation of the products obtained by oxidizing pyrolysed truxene with nitric acid. The main effort of the physical chemistry laboratory was in studies of the mechanism of thermal decomposition of coal and the control of the decomposition by gaseous and gas-entrained additives; but the effects of ultrasonic irradiation on small molecules were also examined and the viscosity characteristics of solvent extracts from coal and the shape and size

of the extracted molecules. In palæobotany, substantial progress was made in formulating means for identifying fossil spores and pollen grains, and the development of a 'key' for identifying living and fossil conifer woods is now virtually complete. Further studies are reported on fluidized carbonization, and the first stage of a coal grindability investigation was completed. In the petroleum division studies continued of the catalytic desulphurization of sulphur compounds at high temperatures to remove sulphur as hydrogen sulphide and on the oxidation of sulphur compounds to remove it as sulphate. The study of two-phase flow of oil and water in pipe-lines was continued, in which a general mathematical analysis was developed for two immiscible fluids flowing between wide parallel plates and flowing concentrically in a circular pipe. Considerable progress was made in the study of the effects of hydrogen isotopes on the rates of chemical reactions, while the study of the retention volumes of hydrocarbon gases on chromatographic columns of a series of activated charcoals has been extended to an examination of the relative widths of the chromatographic bands. The autothermic surface combustion reactor for pyrolysing hydrocarbons was further developed. A list of publications of the Council is appended.

BIOLOGY OF *AMŒBA*

THE late Robert Chambers was a much-loved personality on both sides of the Atlantic. A publication by the New York Academy of Sciences has been greatly influenced by him, many papers being presented by his former students and 'second generation' of students*. The papers deal exclusively with the 'fission cycle' of the life-history of Amœba. I first made acquaintance with Chambers when studying *Daphnia pulex*, each having worked out its spermatogenesis. Later, I sent to him supplies of *A. proteus* at various localities.

An article by Mazia in "Science in Progress"[1] prepared readers for some of the many good things in this volume. After a short biography of Chambers and an introduction by Hirshfield, the subject-matter is grouped into four parts. In part 1 ("Structural and Taxonomic Considerations"), Torch describes the cytology of Pelomyxa. The most interesting conclusion is that crystals are a metabolic waste product, probably an accessory mechanism for the excretion of nitrogen. Particulates of Amœbæ are studied by Kassel using a drop-retraction technique; proteins on reaching an experimentally introduced oil–water interface unfold and produce a surface denaturation curve resembling that of a medium containing a protein of low molecular weight. Useful practical hints and photographs of apparatus, as well as electron microscope studies, are given in "Microscopic Studies" by Borysko and Roslansky. Beautiful electron microscopic work by Pappas reveals the astounding presence of fine fibrous extensions on the outer surface of the plasmalemma of three species of amœba, and other unsuspected structures. Kudo gives a welcome résumé of the work of the early observers and their nomenclature and makes a strong plea for the retention of the name *Amœba* for the genus.

In part 2 ("Physical Studies and Cell Division") Landau deals with sol–gel transformations in Amœbæ, and considers that the findings of myosin-like proteins in amœboid forms lend credence to the idea of a contractile substratum. "Synchronization of Cell-Division" by James gives much fascinating detail of the observations made during the establishment of the main thesis. Three authors describe pinocytosis, which was discovered by Lewis, and observed by Mast and Doyle in amœbæ, but only recognized as important in the past few years. Holter, in the next paper, gives some beautiful pictures of the phenomenon, and stresses its importance in the physiology of amœboid cells, though he thinks that the original definition may require modification towards less emphasis on the fluid uptake and more on the dissolved substance.

A very long paper of sixty-three pages by Guthwin and Kopac is a vade-mecum for the microscopic enzyme chemistry of carboxylic esterases in Amœbæ. "Cytochemical Differentiation in Normal and Starving Amœbæ", by Heller, is the second article of part 3. An interesting analysis of the cytoplasmic inclusions deals with refractive bodies. As I have repeatedly pointed out, these refractive bodies are nutritive, and for that reason Dr. Carmela Hayes renamed them 'nutritive spheres'. They play a great part in the formation of the spores in the Proteus group of Amœbæ. Their diameter is indicative of the age of the Amœba—an individual with large nutritive spheres is old and ready to sporulate. Cohen, in "Physiological and Morphological Observations", gives the first hint as to the great weakness of the work under review when he says, "*A. proteus* in our experience consists of at least two strains . . . investigators should give the history of the stock they use". I maintain that two distinct species masquerade under the specific name *proteus*. I have had an opportunity of studying a rich culture from the laboratory of Brachet: it was *A. lescherae* and not *A. proteus*. It also contained very young stages of development, proving that even under rigid subculturing a few individuals escape and sporulate. Want of space prohibits more than mention of "Tracer Studies in Amœbæ" by Plaut, "Effect of Selected Chemical Agents on Amœbæ" by Zimmerman.

In part 4 Hirshfield discusses "Nuclear Control of Cytoplasmic Activities" and Prescott pictures the wonderful Cartesian diver for weighing Amœbæ in his article on microtechnique in amœbæ studies. "Micrurgical Studies on Irradiated Pelomyra", by Daniels, is followed by an account of the celebrated work of Danielli on strains of Amœbæ that have been continuously cultivated for years in King's College, London. In conclusion, one would urge the examination of older Amœbæ for the presence of deoxyribonucleic acid. Brachet's beautiful work on the "Cytoplasmic Dependence in Amœba" evidently omits this. The volume ends on a lighter vein when Kopac visualizes Amœba research in 2158. Used in conjunction with Jepps's "The Protozoa, *Sarcodina*"[2], this volume is a useful reference book for all students of *Amœba*.

MONICA TAYLOR

* Annals of the New York Academy of Sciences. Vol. 78, Art. 2: The Biology of the Amœba. By Henry I. Hirshfield and 22 other authors. Pp. 401–704. (New York: New York Academy of Sciences, 1959.) 4.50 dollars.

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VALUE OF CONTOUR ANALYSIS IN EQUATORIAL METEOROLOGY

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SINCE November 1958, daily contour analyses have been made at the East African Meteorological Department, Central Forecast Office, East Africa, for standard pressure-levels from the surface to 100 mb., and for an area which includes all Africa, and Europe to 55° N. Such analyses are believed to be unique for Africa: they are giving an insight into mechanisms governing equatorial weather which has not been provided by conventional streamline analyses of the upper winds.

Flow and changes in the flow are as dependent in the tropics as elsewhere on the pressure field and its evolution. However, the want of radiosonde data, lack of success in explaining day-to-day variations in weather in terms of the surface pressure field, and the absence of a fixed relation between pressure gradient and wind, have led to a concentration in the past decade on streamline analysis alone. Streamlines define usefully the instantaneous wind but they have failed in everyday use in East Africa to account satisfactorily for the observed weather, and they have contributed little by themselves to the solution of forecasting problems. Improvements in radiosonde and radar wind coverage over Africa, due largely to the stimulus of the International Geophysical Year, now permit some deductions regarding the role of the upper pressure distribution to be made.

Principal points of contrast with middle-latitude upper patterns are: (*a*) equatorial pressure gradients are weak, a consequence of the inability of other terms in the equations of motion to balance a large pressure force in the free atmosphere; (*b*) pressure systems which influence East African weather are slow-moving or stationary and weather changes are effected more often through development *in situ* than by travelling disturbances.

Though the observations are still insufficiently dense and accurate to define the contours precisely, certain characteristic states have been recognized. An important case arises when upper anticyclones in the lower latitudes of each hemisphere are separated by a trough along the equator (Fig. 1). This system is associated with zonal flow. Pressure gradients and winds derived by assuming a geostrophic balance agree well with observations. When the contours are confluent as in the right of Fig. 1, acceleration leads to convergence near to the equator, and if the convergence extends in depth through the lower layers, a rain-producing mechanism exists. Understandably, the most marked effects on the weather occur when the pressure field is not stationary, but either or both of the anticyclones change their intensity or location; the accelerations are then potentially greatest since the geostrophic balance is particularly delicate due to the smallness of the Coriolis parameter.

A second well-defined model occurs when a meridionally directed pressure gradient exists across the equator (Fig. 2). The geostrophic departure increases as the equator is approached and the unbalanced pressure force at the equator itself leads simply to down-gradient (Eulerian) flow: the contours are crossed perpendicularly by the streamlines a few degrees downstream of the equator, the lag being due to the zonal momentum carried. This model was observed primarily in the period January–March 1959 when, in the lower troposphere, the low pressure to the south took the form of a zonal trough. Cochemé[1] has remarked on the predominance of this pattern near the surface during February 1955 and has noted the resemblance to the large-scale monsoon flow associated with the Indian summer low pressure. On other occasions the low pressure is the equatorward portion of a large-amplitude trough in the polar westerlies which has penetrated the subtropical anticyclonic belt. This pattern was typically associated with lower divergence and fine weather north of the equator and with rain in the convergent westerlies to the south.

The foregoing two cases have been illustrated in simple form; but smaller-scale complications, some orographic, some developmental, may be superimposed upon them, and the two patterns can co-exist in adjoining longitudes. Other characteristic states arise. Of particular importance are those which give rise to westerlies in the lower half of the troposphere. East African meteorologists have long recognized an association between westerly winds and widespread rain. Previously this has been ascribed to the presence of a source of moisture to the west. We observe, however, that a more than adequate supply of moisture is carried inland in the lower layers of the easterly monsoon currents, and consider this to be the more important moisture source for East African rainfall. Westerly flow is often simply

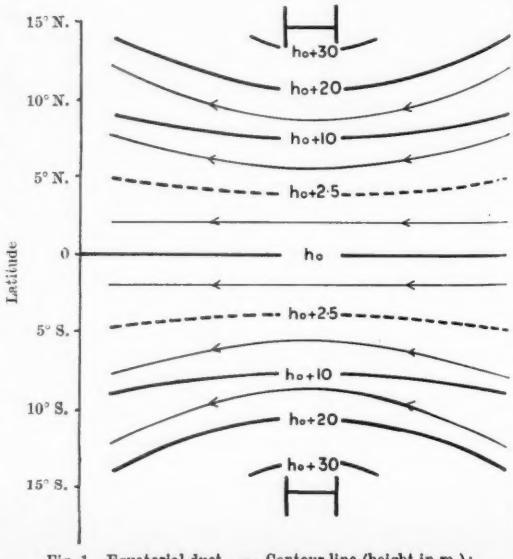

Fig. 1. Equatorial duct. —, Contour line (height in m.); —·—, streamlines

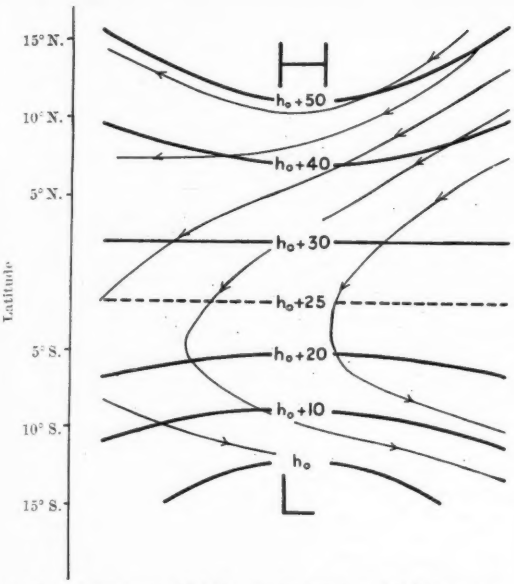

Fig. 2. Cross-equatorial drift. —, Contour lines (height in m.); →—, streamlines

a recurving easterly monsoon current. A basic tendency for moisture to be contained in the lower layers in easterlies, due to subsidence, and for upward transport of the moisture in westerlies, is ascribed to the operation of the Coriolis term, $2\Omega\cos\varphi.u$, in the equation for the vertical acceleration :

$$\frac{dw}{dt} = -\alpha\frac{\partial p}{\partial z} - g + 2\Omega\cos\varphi.u$$

where the symbols have their conventional significance. Considering a fixed value for u, the term $2\Omega\cos\varphi.u$ has its maximum at the equator. It has usually been dismissed as being negligibly small[2] in comparison with the pressure gradient and gravity terms, but these oppose each other, being approximately in balance. In these circumstances smaller terms can achieve significance. Calculation of the last term shows that, providing the possible compensating mechanisms only partially diminish the instantaneous acceleration, appreciable vertical motions could build up on the time-scale of synoptic processes. The effect is by no means over-riding and may be opposed or enhanced by the synoptic situation.

The importance of examining pressure and flow patterns at several levels of the lower troposphere when assessing the significant vertical motions was amply demonstrated during May 1959. Marked low-level divergence over Kenya and Tanganyika, in the outflow from the surface/850 mb. Mauritius/Madagascar sub-tropical anticyclone, underlay on different occasions a confluent 700 mb. pattern (Fig. 1) and a weak westerly flow ; in both cases no rain fell.

As recently as December 1958, the opening speaker at a Meteorological Office discussion on tropical meteorology[3] remarked that the forecasting of upper winds from prontours is invalid in equatorial regions because a relationship between contours and wind has not been established. Contrariwise, the present work, which raises hopes of a basically synoptic solution to East African weather forecasting problems, suggests that the ability to predict changes in the contour pattern in low latitudes is a fundamental need. We most strongly disagree with the view expressed by Walker in the same discussion[3] that there is no guarantee that an improved upper air network would improve (equatorial) forecasting significantly. In a paper which has very recently come to hand, Palmer and collaborators[4] have proved the occurrence of approximately geostrophic flow down to equatorial latitudes in the Pacific Trades ; their investigation demonstrates most eloquently the value of a close homogeneous network of radiosonde and radar wind observations in solving the problems of equatorial flow : the establishment of a similarly close network in East Africa and adjoining territories, where a greater variety of pressure patterns and far more synoptic change occurs, would, we contend, lead to fundamental progress in our understanding of equatorial weather mechanisms.

We wish to thank Mr. B. W. Thompson, regional meteorological representative, Kenya, East Africa, for his constant support and advice, and Mr. J. P. Henderson, director, East African Meteorological Department, for permission to publish this account.

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PERMANENT MOISTURE EXPANSION OF CLAY PRODUCTS ON NATURAL EXPOSURE

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THE criticisms by Vaughan and Dinsdale[1] of our communication[2] on the progressive long-term moisture expansion of clay products, and their objection to our use of the term 'permanent' to describe it, lead us to fear that they still regard the problem as one of academic interest only and not as a serious practical one, such as our observations of damage to buildings in Australia[3] and those of McBurney[4] in the United States of America have shown it to be. Although the expansion may apparently be removed by subjecting the materials to high temperatures, it is permanent at atmospheric temperatures, and hence as long as we are considering the problem as a practical one

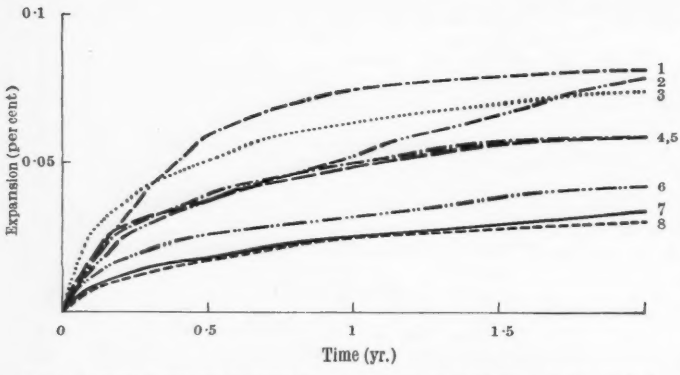

Fig. 1. Average permanent moisture expansion curves for eight types of clay product on standing in air for two years. Curve 1, roofing tiles; curve 2, wall tiles; curve 3, architectural terra-cotta; curve 4, bricks; curve 5, china body; curve 6, refractory body; curve 7, floor tile; curve 8, firebrick

the expansion can properly be referred to as permanent.

Our main object in studying the problem is to determine, for a wide range of clay products, the ultimate expansions and the times taken to reach these, whether they be 50, 100 or 1,000 years. A knowledge of both is necessary if we are to find out how to offset the expansion and so prevent the resultant damage. The reason that we, like others, have autoclaved the bodies in steam has been to accelerate the process of expansion and thus to study, in a reasonable time, the reactions which normally take a life-time or more. Unlike others, however, we have continued the process of autoclaving until the bodies reached dimensional stability and have thus obtained measures of the maximum expansions the bodies suffer at high temperatures. At the same time, we have not neglected to observe the bodies under natural conditions, for it is only thus that the practical behaviour of the bodies can be correlated with the results at high temperatures.

In all, we now have more than a thousand industrial and laboratory specimens exposed under natural conditions, and these are being added to from time to time. Replicate samples are exposed, out of doors, in an air-conditioned laboratory and in an atmosphere of 100 per cent relative humidity, while others are kept totally immersed in water; others again have been subjected to cycles of soaking in water and drying at 110° C. Some bricks have now been under observation for five years, at the end of which time they show expansions ranging from 0·045 to 0·20 per cent with an average rate still between 0·005–0·006 per cent per annum. Most products have been under observation for shorter periods, and the average and range of the expansions for the eight types of body for which results on autoclaving have already been reported[2], after standing for two years either in the laboratory or out of doors, are given in Table 1. Curves for the average expansion of the products with time are given in Fig. 1. Because of the small number of some of the products so far examined the values cannot be considered as completely characteristic, but they do indicate the general trends for the various bodies.

Our results show that the expansions on standing in air are higher than those predicted from the temperature coefficient of the expansion process based on high-temperature data[3], and from this and other observations it is clear that there are no practical relationships between autoclave and low-temperature data[3]. Estimates of ultimate expansions likely in practice and the times to reach these have therefore to be based on the results obtained for natural exposures to date, and as they come to hand in the future.

From our experience we are not surprised that specimens used by Vaughan and Dinsdale did not reach equilibrium during relatively short periods of either natural exposure or autoclaving. Standing for three years is a long way short of the periods for which we know that bodies can continue to expand[3], and calculations based on the temperature coefficient of the expansion process[2,3] show that times of the order of 12,000 hr. would be required if the bodies are to reach dimensional stability in steam at 50 lb./sq. in.

We have observed shrinkages on drying as reported by Vaughan and Dinsdale only in under-burnt bodies, and then only on drying after more than one cycle of wetting and drying; these shrinkages are, however, always less than the original expansions on wetting. Normal ceramic bodies have, in general, continued to expand on drying at 110° C. whether they have been soaked in water or treated in the autoclave[3]. This has also been the experience of other workers[5], and Bonnell and Butterworth[6] have found that no less than 50 per cent of the bricks of the United Kingdom expanded when tested for drying shrinkage. Only when specimens have been autoclaved until they approach dimensional stability (far beyond the stage to which Vaughan and Dinsdale's specimens were taken) do drying shrinkages become apparent, and then only of the order of 0·01–0·02 per cent, a mere fraction of the total expansion. In this connexion it is of practical significance that all nine experimental walls which have now been standing for more than three years at this Division expanded at slightly higher rates when drying out during the hot summer months[3]. The explanation for this is simple: it is due to the increased rate of reaction between the reactive compounds in the bricks and the water still present at the higher temperatures during the drying out process.

Vaughan and Dinsdale's observation that a complete reversal of the change in size can be obtained by heating to 300° C. suggests that they have not investigated a very wide range of bodies. This

Table 1. Permanent Moisture Expansion for Eight Types of Clay Product standing in Air

Product	No. examined	Expansion	
		Average (per cent)	Range (per cent)
Roofing tile	94	0·082	0·162–0·009
Wall tile	18	0·079	0·094–0·066
Architectural terra-cotta	12	0·074	0·098–0·059
Brick	260	0·061	0·186–0·010
China body	6	0·061	0·110–0·034
Refractory body	6	0·042	0·080–0·023
Floor tile	12	0·034	0·080–0·014
Firebrick	12	0·031	0·045–0·020

phenomenon has been reported once before[7], but most other workers have found that temperatures of at least 600° C. and often much higher are necessary for this purpose[8]. In some cases it has proved impossible to remove expansion without heating to temperatures approaching and above those at which the specimens were originally fired. At these temperatures further firing shrinkages occur and the two effects become confused; this suggests that even at lower temperatures the shrinkages obtained by reheating are not necessarily caused by a reversal of the expansion process, and hence any approximate numerical agreement may be quite fortuitous.

Our critics also raise the question of expansion taking place during the cooling of the clay bodies in the kiln, and its effect on the establishment of a standard zero of measurement. We are fully aware of this problem but do not see that their proposal of a preliminary desorption is of practical value. The specimens will still have to be cooled after this treatment before they can be measured, and hence they will again adsorb moisture, and further, as we have pointed out above, most bodies if heated after they have adsorbed moisture expand still more. We therefore consider that the small amounts of expansion in the kiln must be disregarded from the practical point of view. What matters is the expansion that takes place in the structures into which the units are built, and therefore the practical basis for calculating and comparing expansions is the ex-kiln length of the products.

From experimental evidence in this Division[2,3,9] we consider that chemical processes involving hydration of such constituents as amorphous alumino-silicates, glasses and amorphous silica are responsible for expansion, and that reversible physical swelling, except in under-burnt bodies, plays a very minor part. Furthermore, we consider that the evidence presented by our critics does not substantiate a physical explanation.

A full account of our work to date on bricks[3] is now available and we hope to present a summary of current studies on the expansion (under natural conditions of exposure) of all clay products examined so far to the seventh International Ceramic Congress in 1960.

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EXISTENCE OF AN INNER AURORAL ZONE

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FROM theoretical considerations Alfvén[1] predicted the existence of an inner auroral zone at a polar distance of 5–10°. Nikolsky[2], Meek[3], and others have found that the maxima of geomagnetic and ionospheric disturbances are concentrated around a spiral-shaped zone which Nikolsky identifies with the inner auroral zone. However, no observation of auroræ in relation to this zone seems to have been made; the spiral-shaped zone is presumably identical with the curve of geographical distribution of maxima of magnetic activity of class J[4]. At Godhavn (69·2°; 306·5° geog.) this class of activity is not at all, or very poorly, correlated with auroræ; hence the zone of Nikolsky, Meek and others can scarcely be regarded as an auroral zone in the proper sense.

It is the purpose of this article to direct attention to a 'population' of auroræ which seems to form an inner auroral zone; it summarizes a study which was presented in part at the Auroral Conference at Uppsala in August 1958. A more detailed paper will be published elsewhere.

As a result of visual auroral observations at Godhavn during 1952–56 it was found that the diurnal distribution of auroral frequency there had two maxima. In fact, the distribution is composed of two distributions of different types. One, with a maximum near magnetic midnight, is formed by relatively brilliant auroræ, approaching and sometimes passing zenith from the south-east, accompanied by negative magnetic bays. These auroræ are especially frequent and brilliant on international disturbed days and in years with low sunspot activity.

The second distribution with a maximum at about 6h. local time is formed by zenithal auroræ. These are narrow diffuse bands, sometimes with a faint ray structure, or draperies—often several parallel draperies—composed of long rays that may be at some distance from each other. The draperies may form fans or coronas, occasionally accompanied by colour effects, but generally the movements of the arcs and rays are very moderate. They may rather be described as slow pulsations of intensity; narrow homogeneous arcs slowly die away, while parallel arcs a few degrees from them grow in intensity. These slow variations may go on for a long time; they seem quite irregular and not in phase in the different arcs or rays, so that the mean position of the aurora is little affected by the intensity variations.

Several authors have assumed that the main auroral zone is displaced polewards in the morning hours. There is no observational evidence that this is the reason why auroræ are observed at Godhavn in the morning hours. On the contrary, in the cases where the onset of the morning-type auroræ was observed, very weak auroræ began sporadically at a great elevation at about 3h.–4h. (Ascagrams from the International Geophysical Year, too, show that the auroræ begin suddenly within a few degrees from Godhavn.) The mean elevation did not change significantly in the course of the morning; the mean distribution of the elevation of the arcs and draperies had its maximum a little to the south of zenith (Fig. 1).

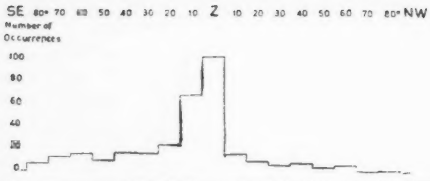

Fig. 1. Frequency-distribution of zenith-distances, Godhavn, 1954–56

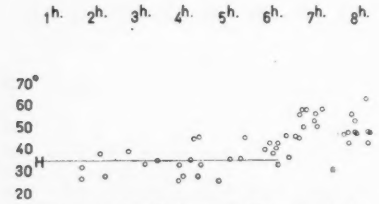

Fig. 2. Azimuth of arcs, Godhavn, 1954–56

The direction of the bands was perpendicular to *H* until 6h.; at about 6h. the mean direction suddenly changed in a clockwise sense (as seen from above). The change is consistent with Alfvén's theory[5]. The order of magnitude was 20° in 1954–56 (Fig. 2).

Whereas the evening-type auroræ are strongly related to magnetic activity, the morning-type auroræ are not correlated with local magnetic activity, and they appear to be independent of the planetary state of disturbance, too. Thus, observations from Upernavik, a few degrees to the north of Godhavn, from the years 1884–1937, show that auroræ noted at 21h. L.T. were eight times as frequent on international disturbed days as upon international quiet days. Auroræ noted at 8h. were here, as well as at Jakobshavn, a hundred kilometres to the east of Godhavn (observations 1885–1915), distributed at random between quiet, disturbed and the remaining days. At Godhavn auroræ were observed on 106 of a total of 110 clear mornings without moonlight in the winters 1954–55 and 1955–56.

A study of published auroral reports from arctic expeditions has shown that the morning-type auroræ observed at Godhavn can be found at several other locations too. Thus the auroral type mentioned was observed near zenith at Kingua-Fjord[6], and it seems likely that the same was the case at Chesterfield[7,8], Coppermine[7] and Murchison Bay[9]. Further, auroræ near zenith in the morning hours were observed at Danmarkshavn (76·5°; 341°), Station Nord (81·5°; 342°) and at a number of stations on the western coast of Greenland not very distant from Godhavn (unpublished observations, Danish Meteorological Institute).

To the south of Godhavn the auroræ seem to have been observed from Godthåb, whereas at stations in southern Greenland nearer to the main auroral zone this type of aurora has not been identified.

At stations nearer to the centre of the auroral isochasms (Peary Land, Upernavik, Thule) the morning auroræ are observed less frequently and mainly on the southern sky (unpublished observations).

In the Antarctic the inner zone auroræ were observed by several expeditions, for example, by Mawson at the stations Cape Royds and Cape Denison[10].

On the map in Fig. 3 (after Hultquist[11]) the places at which the morning auroræ are observed near zenith have been plotted as circles. They are seen to form a zone which is practically coincident with Hultquist's auroral isochasm through Godhavn. This oval, which is the projection of a circle in the equatorial plane outside the Earth on to its surface along the geomagnetic lines of force, may thus be regarded as an inner auroral zone, occupied in the early morning- and day-hours of all days, irrespective of disturbances in the main auroral zone.

Fig. 4 shows the diurnal distribution of auroral frequencies for December and January 1948–50 at five stations in Greenland. The stations are Thule

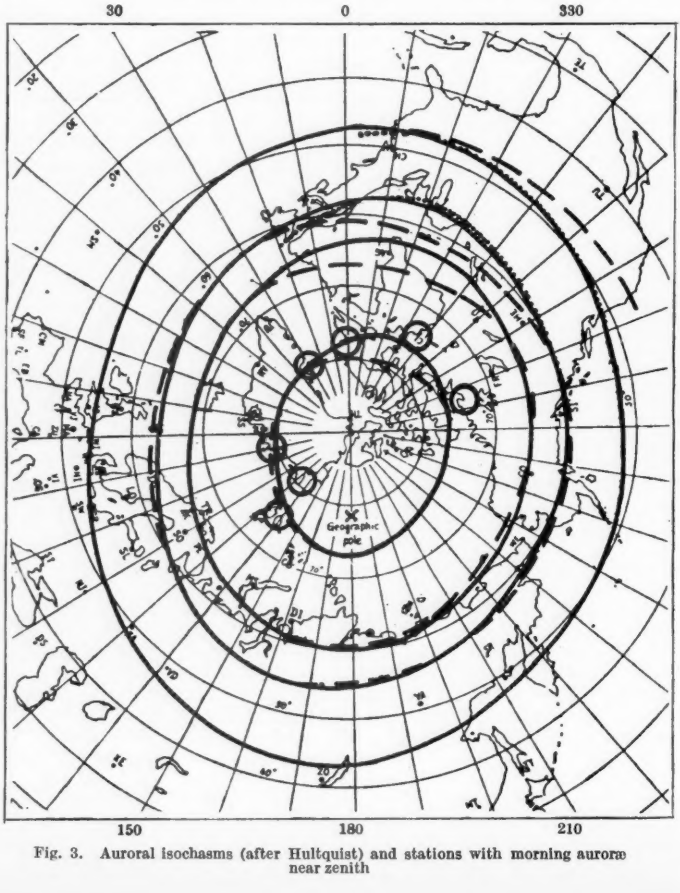

Fig. 3. Auroral isochasms (after Hultquist) and stations with morning auroræ near zenith

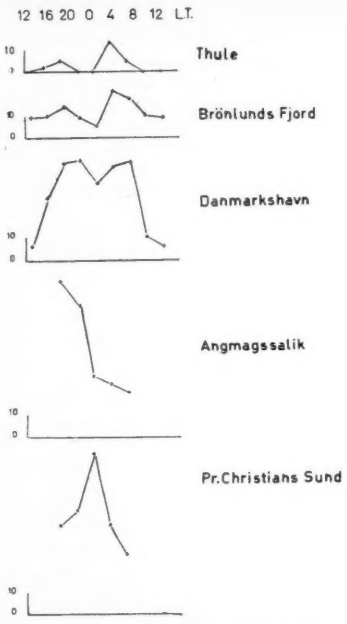

Fig. 4. Percentage of clear hours with aurora at five stations in Greenland, December and January 1948–50

(76° 33′ N.; 68° 49′ W.), Brønlunds Fjord, Peary Land (82° 10′ N.; 30° 30′ W.), Danmarkshavn (76° 46′ N.; 18° 45′ W.), Angmagssalik (65° 36′ N.; 37° 33′ W.), and Prins Christians Sund (60° 03′ N.; 43° 12′ W.). The figure gives the percentage of clear hours with aurora, arranged according to local time; the same scale has been used at all stations. Whereas the frequency of evening auroræ increases from Thule to Angmagssalik–Prins Christians Sund it is clearly seen that the frequency of the morning auroræ has its maximum at Danmarkshavn, in accordance with the idea of the existence of an inner auroral zone there.

Hulburt[12], in his study of the diurnal variation of auroral frequencies, takes the view that "Vegard's conclusion, that most auroral forms show an evening and morning maximum, is contradicted almost as often as it is upheld". From Fig. 4 (which is in agreement with material from several other stations) it may be seen that the different types of diurnal distributions can be arranged according to the following scheme: (*a*) stations at the main auroral zone have distributions with a single maximum near geomagnetic midnight; (*b*) stations between the zones have often, in addition, a weak morning maximum; (*c*) stations near the inner auroral zone show two maxima, one near geomagnetic midnight and one at about 6h. L.T.; (*d*) stations some degrees of latitude nearer to the pole show a weak geomagnetic midnight maximum and a distinct morning maximum.

Great ionospheric disturbances begin at the same time as the onset of the morning auroræ. The *F*-layer is dissolved into spread *F* and oblique incidences, which are soon accompanied by sporadic layers of auroral type at different virtual heights between the *E*- and *F*-layer heights. Whereas observations of auroræ are restricted by cloud and daylight, a study of ionospheric disturbance is possible at any time. The occurrence of ionospheric disturbance may be represented by the mean daily distribution of the frequency of $E_s > 3$ Mc./s., which is published monthly by the National Bureau of Standards. By combining the frequencies for 3h.–8h. L.T. for the years 1952–56 it was found that the yearly variation of the morning disturbances is a single wave with a maximum at midwinter and a minimum at midsummer. The variation through the whole period seems to indicate a variation in phase opposite to the sunspot variation.

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ISOMERS OF VITAMIN A IN FISH LIVER OILS

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WE have investigated the occurrence of the '*iso-a*' and '*iso-b*' isomers of vitamin A in a number of fish liver oils by means of reaction with the retinal protein, opsin. These isomers were found to constitute about 20 per cent of the vitamin A in cod, shark and mixed fish liver oils examined. Since the presence in fish liver oils of all-*trans* vitamin A and neovitamin A (*neo-a*)[1] has previously been reported, all four of the 'unhindered' isomers have been found in these oils.

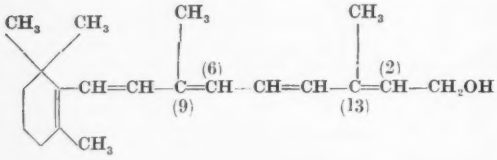
The finding assumes importance because the *iso-a* and *iso-b* isomers have only about one-fourth the growth-promoting activity of all-*trans* vitamin A, as measured by rat bioassay[2].

Nomenclature, structure and biopotencies for the *iso-a* and *iso-b* isomers are shown in Table 1.

The trivial names devised by Hubbard and Wald[3] are used in this report because official action on a numbering system has not yet been taken. The numbering systems now used include (*G*) Table 1,

Table 1

Trivial name	Stereoisomeric form	Relative biological potency (ref. 2) (per cent) Vitamin A acetate	Vitamin A aldehyde (retinene)
	all-*trans*	100	91
	C *G*		
iso-a	9-*cis* 6-*cis*	21	19
iso-b	9,13-di-*cis* 2,6-di-*cis*	24	17



the Geneva system used by *Chemical Abstracts*[4] and employed in earlier[2,5] and forthcoming papers from the Distillation Products Industries Laboratories and (*C*) the system analogous to that used with the carotenoids and employed by Wald, Brown, Hubbard and Oroshnik[6].

Hubbard and Wald[7] first reported the presence in a concentrate from fish liver oils of a *cis*-isomer of vitamin A different from the neovitamin A found by Robeson and Baxter[1]. Later studies by Hubbard and Wald[3] on pure retinene (vitamin A aldehyde) isomers synthesized in the Distillation Products Industries Laboratories[5,8] showed that the isomer in fish liver oil was *iso-a* vitamin A. The basis for the identification was the observation that both *iso-a* retinene and the retinene corresponding to the *cis*-isomer formed the same pigment with opsin, namely, *iso*rhodopsin, having an absorption band maximal at 487 mμ. *Iso-b* retinene, on incubation with opsin, slowly isomerizes to *iso-a* retinene and forms the same pigment.

This reaction was afterwards developed by Hubbard, Gregerman and Wald[9] as an analytical method for determining the *iso-a* and *iso-b* isomers in the absence of *neo-b* retinene. The *neo-b* isomer, the key isomer in the visual process, interferes in this reaction by yielding the pigment rhodopsin, absorbing at 500 mμ. The interference from *neo-b* retinene has not complicated studies on fish liver oils because this isomer has not yet been found outside the eye[10].

The results of applying this analytical procedure and an infra-red method to vitamin A preparations are given in Table 2.

Four fish liver oil preparations (shark 'non-sap', that is, the non-saponifiable fraction, before and after concentration by molecular distillation; cod 'non-sap' and the 'non-sap' from distilled cod liver oil) were prepared (by M. H. S.) and assayed by the opsin method (P. S. B.). The test samples were saponified and the vitamin A isomers were recovered by extraction (U.S.P. XV method). Sterols were separated by crystallization from methanol at −20° C. The isomers were oxidized to the retinenes by procedures similar to those of Wald[11] with manganese dioxide prepared according to Henbest, Jones and Owen[12]. The cod liver oil was a pharmaceutical preparation from an apothecary; the shark liver oil was from a bulk commercial shipment obtained at Distillation Products Industries. Table 2 shows that 19–26 per cent of combined *iso-a* and *iso-b* isomers were present in the vitamin A of the oils.

After further refinement of the procedures, the vitamin A of a pharmaceutical grade, mixed fish liver oil (sample 5, Table 2) was found to contain 19 per cent combined *iso-a* and *iso-b* isomers.

The content of combined *iso-a* and *iso-b* vitamin A in a sample of oil from rat livers (sample 6, Table 2) was examined. The value found (14 per cent) was lower than that found for the fish liver oil preparations examined, but was substantial and indicates that these isomers occur in other oils besides fish liver oils.

The origin of the *iso-a* and *iso-b* isomers in fish liver oils is uncertain. Ames, Swanson and Harris[13] have suggested, on the basis of bioassays of rat liver oils, that the isomers originate there from *in vivo* isomerization of all-*trans* or neovitamin A to form an equilibrium mixture. A similar isomerization may occur in fish liver oils.

Another possibility is that the '*iso*' isomers may sometimes be formed during processing of fish livers. Lambertsen and Braekkan[14], for example, have reported evidence suggesting that neovitamin A is formed from all-*trans* vitamin A during commercial processing and it is possible that '*iso*' isomers may also be formed. Pharmaceutical grade oils were used in the present study, where available, to ensure that the samples used had received as careful commercial processing as possible.

The saponification and oxidation procedures used in the present work to prepare the samples for assay were examined as a source of isomerization. Control experiments in which all-*trans* vitamin A palmitate was so processed produced negligible amounts of '*iso*' isomers, as measured by the infra-red procedure, and ruled out this possibility.

The recognition of the prevalence of the *iso-a* and *iso-b* isomers made it of interest to isomerize samples of retinene *in vitro*, with dilute hydrochloric acid, until they had attained a state of 'equilibrium', defined as the point at which further exposure to acid produced no change in the proportion of the four isomers present. Assays by the opsin and infra-red methods are reported in Table 2. As in fish liver oils, the proportion of combined *iso-a* and *iso-b* isomers in the vitamin A of the artificial iso-

Table 2

Sample No.	Description*	Percentage *iso-a* + *iso-b* in total vitamin A isomers (opsin assay)	Percentage *iso-a* + *iso-b* in total vitamin A isomers (infra-red assay)
	Liver oil concentrates:		
1	Shark 'non-sap' E(1%, 1 cm.) (328 mμ) = 538	24	—
2	Shark distilled 'non-sap' E(1%, 1 cm.) (328 mμ) = 628	26	—
3	Cod 'non-sap' E(1%, 1 cm.) (328 mμ) = 202	19	—
4	Distilled cod 'non-sap' E(373 mμ) = 427	25	—
5	Mixed fish liver oil 'non-sap' E(373 mμ) = 350	19	—
6	Rat liver oil† 'non-sap' E(370 mμ) = 270	14	—
	'Equilibrated' retinene isomer concentrates:		
7	E(372 mμ) = 1,030	21	18
8	E(377 mμ) = 1,260	23	22
9	E(374 mμ) = 1,080	20	21

* The vitamin A in samples 1–6 was oxidized to retinene by manganese dioxide prior to assay.

† Oil was a portion of that kindly supplied by Dr. T. K. Murray, Food and Drug Directorate, Ottawa, Canada, for related bioassay studies.

merates was about 20 per cent. The assays were run independently and the results indicated that with preparations of the purity investigated the two methods are in good agreement.

The average ratio of *iso-a* : *iso-b* isomers in the equilibrated samples was found to vary from about 2 : 1 to 4 : 1. This variability is probably caused, in part, by errors in measuring the relatively small amounts of *iso-b* isomer present.

Further information on the experimental methods is as follows.

Opsin. Opsin was prepared in digitonin solution, using a modification of the procedures described earlier[3,9,10]. This procedure will be reported in detail separately.

The essential steps in this procedure included dissection of retinæ from cattle eyes in the light, disintegration of the tissue by grinding, floating out the fragments of outer segments of the rods by differential centrifugation in phosphate buffer weighted with 40 per cent sucrose; lyophilization of the rod fragments, and exhaustive extraction of the dry powder with petroleum ether to remove extraneous lipids and vitamin A; and finally the extraction of opsin from the residue with 2 per cent aqueous digitonin. Such preparations were assayed for their opsin content with *neo-b* retinene (ref. 10). They were stored at about −15° C. until used for assay. They remained stable for many months.

Assay of retinene isomers. Samples of the mixed retinene isomers in a few drops of ethyl alcohol were pipetted into 2–3 ml. of 2 per cent digitonin in water. A cloudy solution usually resulted, depending on the concentration, but this was cleared by filtering through a fine sintered glass filter. The final solutions used for assay contained 5–10 μgm. of retinene.

Such solutions were assayed with opsin for their combined content of *iso-a* and *iso-b* isomers, as described for the assay of opsin itself, except that at least three times the equivalent amount of opsin was used in the determination. The photosensitive pigment formed in all experiments was *iso*rhodopsin ($\lambda_{max.}$ = 487 mμ), not rhodopsin.

Iso-a retinene combines directly with opsin to form *iso*rhodopsin. *Iso-b* retinene slowly isomerizes to *iso-a*, and hence forms the same pigment. With *iso-a* retinene, this synthesis is complete in 2 hr. at 23° C.; whereas with *iso-b* retinene the same process is completed only within about 24 hr.

One therefore incubates an unknown mixture of retinene isomers with excess opsin, and measures the amount of *iso*rhodopsin formed at 23° C. in 2 hr., and in 24 hr. The amount formed in 2 hr. is primarily formed from *iso-a* retinene; that formed in 24 hr. measures the combined amounts of *iso-a* and *iso-b* retinene. A small correction of the 2-hr. value is needed to take care of the very small amount of *iso*rhodopsin formed from *iso-b* retinene during this period; the 24-hr. value is also corrected for the very small, almost negligible, synthesis of *iso*rhodopsin by the isomerization of all-*trans* and *neo-a* retinene during the incubation period.

Total retinene. The total amount of retinene in the samples was estimated by the antimony chloride reaction and recorded with a Cary recording spectrophotometer. The amount of retinene is determined on the basis that this blue product has E(1 per cent, 1 cm.) (664 mμ) = 3,740. From this and the preceding data, the percentage of combined *iso-a* and *iso-b* retinenes in the total retinene is computed.

Infra-red procedure. This work (by W. P. B.) was based on the curves for the individual retinene isomers already reported[5]. The percentage of combined *iso-a* and *iso-b* retinenes in the mixed retinene isomers is calculated from the absorption at 8·73μ, corrected for interference from the all-*trans* vitamin A and neovitamin A present by the absorption at 8·48μ.

The infra-red method has the advantage over the one employing opsin, that it can be used to determine the percentage of each of the four isomers in the sample, although we describe here only its use in determining the percentage of *iso-a* + *iso-b* isomers present. It has the disadvantage that the retinene content of the sample needs to be relatively high, at present, to minimize errors due to the absorption of impurities. We prefer a purity of about 75 per cent, E(1 per cent, 1 cm.) (370 mμ) = about 1,000, but procedures for concentrating preparations of lower purity are under study.

The equation employed in the Distillation Products Industries Laboratory is:

$$\text{Percentage } iso\text{-}a + iso\text{-}b \text{ in mixed isomers} = \frac{100\,k_a - (k_b + 0{\cdot}01)}{k_c \times P}$$

where k_a = the extinction coefficient for the test sample at 8·73μ (1 per cent solution in carbon disulphide, 1 mm. cell, Perkin Elmer, Model 21, instrument, sodium chloride prism, slit programming No. = 9·75); k_b = the extinction coefficient at 8·48μ for the test sample; this value plus 0·01 is the 'background' correction determined with pure all-*trans* and neovitamin A aldehydes in a ratio (3 : 1) which approximates that present in the mixed isomer preparations studied; k_c = the extinction coefficient at 8·73μ for pure *iso-a* and *iso-b* retinenes in a ratio (3 : 1) which is approximately that present in the mixed isomer preparations studied; P = the concentration (per cent) of the total retinenes determined by the antimony trichloride reaction.

This work between the two laboratories was initiated and co-ordinated by Dr. J. G. Baxter, Distillation Products Industries, with the co-operation of Prof. G. Wald, Harvard University. Related bioassays and analytical studies by members of the Biochemistry, Vitamin Development and Organic Research Laboratories, Distillation Products Industries, were helpful in guiding the direction taken in the work. These bioassays and analyses will be reported separately.

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LIVER GLUCOSYL OLIGOSACCHARIDES AND GLYCOGEN; CARBON-14 DIOXIDE EXPERIMENTS WITH HYDROCORTISONE

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IT has not been possible for geographical reasons to continue a collaborative study designed to clarify, through the use of carbon-14 dioxide, the biochemical significance of liver glucosyl oligosaccharides, particularly in relation to the action of adrenal corticoid hormones. We therefore wish to report our findings to date.

Sie and Fishman[1] observed earlier that hepatic glucosyl oligosaccharides fluctuated in the same direction as did liver glycogen in starvation, glucose feeding and insulin administration. Under these conditions, our concern lay with the dynamics of the glucose unit, either produced from glycogen, as in starvation or in insulin administration, or exogenously provided in the glucose feeding experiment. On the other hand, liver glycogen resulting from the action of hydrocortisone in fasted rats[2] is considered to originate from the catabolism of protein. Since hexose synthesis from pyruvate and lactate is believed to require carbon dioxide fixation[3], hepatic gluconeogenesis could be followed with carbon-14 dioxide without supplying an exogenous substrate or preformed glucose units. The following experiments have been carried out.

In the first series of experiments, male Wistar rats (150–175 gm.), after 22–24 hr. of fasting, were injected with 50 μc. of carbon-14 dioxide bicarbonate (isotonic saline, intraperitoneally) 1 hr. before killing, and at 3 hr. and 5 hr. after hydrocortisone (5 mgm./animal in saline microcrystalline suspension) was injected by the same route (Fig. 1). In the second series of experiments, 50 μc. of radioactive bicarbonate was injected 3 hr. after an initial injection of 5 mgm. of hydrocortisone. Animals were killed afterwards at intervals up to 3 hr. (Fig. 2).

At the times indicated, the experimental animals were killed by decapitation, and the livers rapidly removed and frozen in a dry ice bath. A 10 per cent homogenate of each liver was prepared with ice-cold water, keeping the homogenizer packed in ice. This mixture was deproteinized by additions of 10 ml. of 5 per cent zinc sulphate and 10 ml. of 0·3 *N* sodium hydroxide solution. After centrifugation, the precipitate was washed twice with 10 ml. of cold distilled water, and the washings and supernatant solution were pooled. The oligosaccharides were adsorbed from this mixture with charcoal, which was then washed with copious amounts of distilled water, and eluted afterwards with hot ethanol[1]. Total glycogen was isolated by Roe's method[4]. Glycogen and oligosaccharides were hydrolysed with hot mineral acid[5], and the glucose was estimated by the Nelson method[6]. Tail vein blood was obtained for the determination of carbon dioxide and glucose[7]. The quantity of carbon dioxide in the blood was determined in the Van Slyke apparatus and was recovered and counted as barium carbonate. Glucose was isolated as phenylosazone[8], which was recrystallized, plated and counted. A Robinson flow counter was used for the radioisotope assays[9].

The following observations are made. Animals whose liver glycogen had been almost completely depleted by fasting not only possessed a significant amount of glucosyl oligosaccharides, but also they exhibited about four times the specific radioactivity found in the glycogen and contained virtually all the labelled hexose. With the stimulation of gluconeogenesis by compound *F*, the specific activity of the glucosyl oligosaccharides diminished, while that of glycogen increased at the 4 hr. interval. In Fig. 2, where it is possible to follow all the events as a function of time after compound *F* and carbon-14 dioxide administration at the times indicated, it required about 1 hr. for the carbohydrates to incorporate the maximum level of carbon-14. In the early part of this process (between 3·25–3·5 hr. after compound *F*) the glucosyl oligosaccharides became more richly labelled than

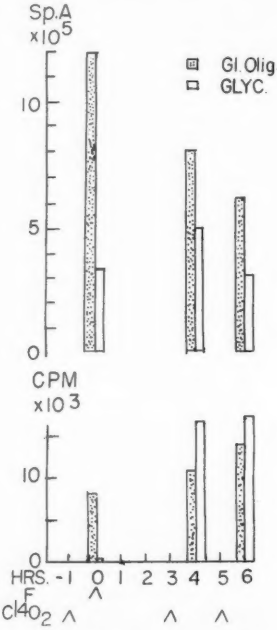

Fig. 1. Carbon-14 labelling of liver glucosyl oligosaccharides and glycogen in relation to the administration of compound *F*. Note that the animals were killed in each instance 1 hr. after the injection of carbon-14 dioxide. The numbers of rats employed for each time interval were, respectively: −1 to 0, 2; 0 to 4, 4; 0 to 6, 2

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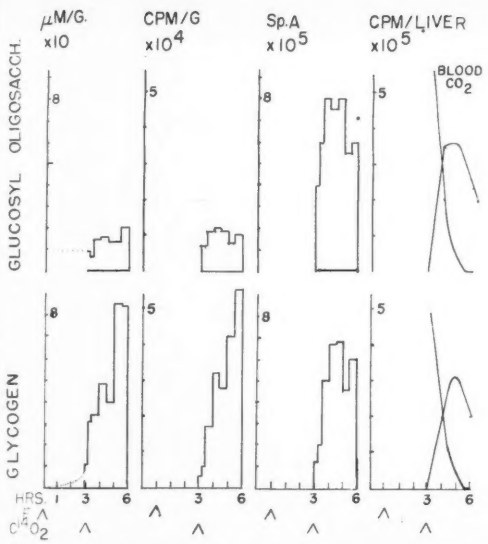

Fig. 2. Carbon-14 turnover in liver glucosyl oligosaccharides, glycogen and blood carbon dioxide in relation to compound *F* administration. The number of animals used was two at each time interval except for the 4-hr. column (4 rats). The relative specific activity of blood carbon dioxide was computed on the assumption that carbon-14 dioxide was incorporated into only carbons 3 and 4 of glucose. The observed c.p.m./mole of barium carbonate have been divided by three, and the same plot (descending curve) appears for both glucosyl oligosaccharides and glycogen

the glycogen by a factor of 3 and for another 2 hr. still remained more radioactive per milligram of polysaccharide glucose. This situation is reflected in the curves relating total counts per liver of each carbohydrate category against a background of rapid carbon-14 dioxide disappearance from the blood. After 3·5 hr. the glucosyl oligosaccharides exhibited a constant number of counts per gm. of liver, whereas those for glycogen continued to rise to the end of the period investigated (6 hr.). However, for this interval, specific activity of glycogen paralleled that for the glucosyl oligosaccharides. In general, carbon dioxide fixation matched carbohydrate synthesis following the injection of hydrocortisone.

The interpretation which appears best to fit the above facts is the following. Pyruvate and lactate (since these fix carbon dioxide during their conversion to glycogen) are probably the major protein-supplied precursors of glucosyl radicals in fasted rats treated with hydrocortisone. These glucosyl radicals are first linked into individual members of a homologous family of glucosyl oligosaccharides (maltose, maltotriose, maltotetraose, etc.) which is 'turning over' rapidly. Under the proper physiological stimulus (glucose feeding, compound *F*) these oligosaccharides are assembled into macro-molecules of glycogen.

In our previous work, first consideration was given to isolating, identifying, characterizing and determining quantitatively liver glucosyl oligosaccharides[10], and the next concern was to rule out the possibility of artefact due to the operation of extraneous factors of either a biochemical or physical nature[1,10]. Our attention was directed recently to the question of the origin of glucosyl oligosaccharides and their possible significance in the metabolism of liver glycogen. The implication of previous experiments[1] (starvation, glucose feeding, insulin administration) and the work of others[11] with rat diaphragm was that glucosyl oligosaccharides (no matter what their origin) may enter somewhere into the mechanism of glycogen synthesis. The present results supply direct evidence in support of this suggestion.

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RELEASE OF NUCLEOTIDES FROM YEAST CELLS

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IT is well known that when *Lactobacilli* are cultured in association with yeast, the former organisms are able to proliferate in a simple medium containing no growth factors specific for them. The resulting growth of *Lactobacilli* is mainly dependent on vitamins, purine and pyrimidine bases secreted by the yeast cells into the medium[1,2]. The present communication contains a description of the ultra-violet-absorbing materials released from the yeast cells and the conditions influencing their release.

Beer yeast (*Saccharomyces cerevisiae*, Yebis) subcultured for 72 hr. at 30° C. in a synthetic medium was incubated for 5 hr. at 30° C. in the same medium containing phosphate-^{32}P. After harvesting and washing three times with distilled water, the labelled cells (dry weight, 1,760 mgm.) were suspended in 400 ml. of 0·08 *M* sodium citrate buffer (*p*H 6·0) with 2 per cent glucose added, and incubated for 3 hr. at 30° C. The supernatants after incubation had an ultra-violet absorption spectrum with a maximum at 258 mμ and a minimum at 235 or 240 mμ.

The ultra-violet-absorbing materials released from the cells were almost completely adsorbed by a charcoal column pretreated with 8 per cent *iso*octanol.

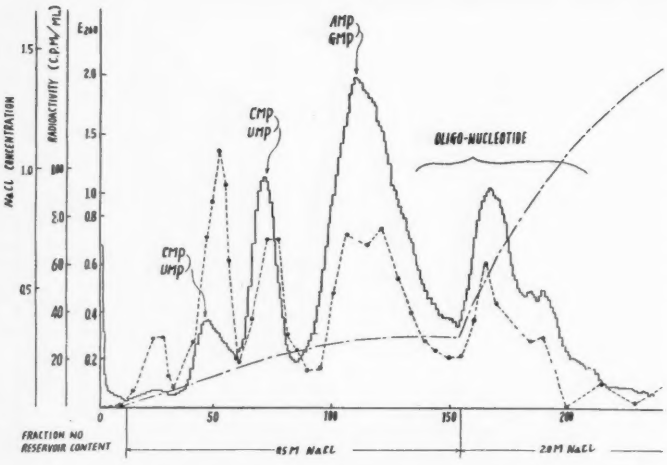

Fig. 1. Separation of the nucleotides released from yeast cells labelled with phosphorus-32 during their incubation in citrate buffer. Column, 'Dowex-2' (chloride), 200–400 mesh, 12 cm. × 0·785 cm.²; elution, the initial mixer content was 500 ml. water, and the reservoir content was 500 ml. of 0·5 *M* sodium chloride; then 500 ml. of 2·0 *M* sodium chloride solution. Each effluent was collected in 4·7 ml. portions with a fraction collector. Sorbed materials, the ultra-violet-absorbing materials prepared as described in text. ———, Optical density at 260 mμ; – – – –, radioactivity (c.p.m./ml.); —·—, sodium chloride concentration in the effluents. CMP, Cytidine monophosphate; UMP, uridine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate

More than 80 per cent of the adsorbed material was eluted with 0·3 per cent ammonia–50 per cent ethanol solution. The eluate from the charcoal was neutralized with 'Dowex 50' (acid type) resin, and then passed through 'Dowex 2' (chloride type) resin. The adsorption in this step was more than 90 per cent. The column was washed with water, and the adsorbed material was separated with a sodium chloride solution by the gradient elution technique[2]. The optical density at 260 mμ and 280 mμ and the radioactivity of each effluent were measured.

The results for six fractions are shown in Fig. 1. The positions, but not the heights, of optical density peaks agree well with those of radioactivity. The initial fractions (eluted with less than 0·3 *M* sodium chloride solutions) were rechromatographed on 'Dowex 1' (formate type) and then on paper; the monophosphates of cytidine, uridine, adenosine and guanosine were identified in this way. The oligonucleotide fractions (eluted with 0·3–1·5 *M* sodium chloride) followed the mononucleotides. The recovery using sodium chloride solution, even up to 4·0 *M*, was only about 60 per cent of the calculated amount of adsorbed material. However, when a mixture of 4·0 *M* sodium chloride and 1·0 *M* sodium hydroxide solutions was used as eluant, the residual material was almost completely separated from the column. This alkali-eluted fraction, the final eluate, contained four mononucleotides, which would presumably be derived from polynucleotides hydrolysed by alkali during the elution. Details of this chromatogram will be published elsewhere.

Oligo- and poly-nucleotides formed more than 60 per cent of the total nucleotides released, and the ratio of purine bases to pyrimidine ones in the mononucleotide fractions was 2·5, though it was 1·2 overall. These ratios depended on the experimental conditions. Most of the nucleotides released were probably fragments of the cellular ribonucleic acid, since scarcely any 5′-nucleotides were in any fraction, and amounts of the nucleotides released corresponded approximately to the decrease of cellular ribonucleic acid. Moreover, the quantity of nucleotide released was greater than that in the pool (fraction soluble in cold acid) which decreased during incubation.

As shown in Table 1, distilled water, sodium chloride and potassium chloride solutions and a synthetic growth medium did not cause a release of nucleotides when used as the incubation medium; acetate or succinate buffers had a slight effect and phosphate or citrate buffers rather more effect. This stimulation of citrate or phosphate might be well attributed to their chelating action, since ethylene-diamine tetraacetic acid, a notable chelating agent, markedly accelerated the nucleotide release, and the addition of *M*/100 magnesium sulphate to *M*/50 citrate buffer resulted in a 50 per cent reduction of the release.

Inhibition of the release in citrate buffer was not observable in the presence of either ordinary antibiotics (penicillin, 20 μgm./ml.; streptomycin, 300 μgm./ml.; and chloramphenicol, 20 μgm./ml.) or of metabolic inhibitors (sodium azide, 10^{-3} *M*; Na_2HAsO_4, 2×10^{-3}; and 2,4-dinitrophenol, 5×10^{-4} *M*), except monoiodo-acetic acid which produced a slight drop at 10^{-3} *M*. The *p*H optimum for the release in citrate or phosphate buffer was 6·0–7·0 and an acid *p*H suppressed the release. Several treatments which disrupt the permeability barrier of yeast cells, for example, freezing and thawing, treatment with organic solvents, and short-time sonication of the cells, stimulated the release considerably. After such treatment an enhancing effect of citrate was also discernible. However, it is possible that the materials released in these conditions are not the same as the nucleotides released by the intact cells, since the supernatant of the citrate buffer incubated with sonicated or frozen and thawed cells showed an increased absorption at 230–250 mμ, which disappeared on acidification with perchloric acid.

Recently, a similar phenomenon has been reported by Holden[4] for *Lactobacilli*. His results, and ours[13],

Table 1. Effect of Incubation Medium on the Release of Nucleotides from the Yeast Cells

All results are expressed in terms of 100 mgm. (dry weight) of the cells

Incubation medium	Released nucleotide-phosphorus (μgm.)
Distilled water	46·5
0·08 *M* sodium chloride solution	33·0
0·08 *M* potassium chloride solution	34·5
0·08 *M* sodium acetate buffer (*p*H 5·0)	65·0
0·08 *M* sodium succinate buffer (*p*H 5·0)	62·0
0·08 *M* sodium phosphate buffer (*p*H 5·0)	80·5
0·08 *M* sodium citrate buffer (*p*H 5·0)	116·0
M/100 Ethylenediamine tetraacetate (*p*H 4·2) solution	68·0
M/100 Ethylenediamine tetraacetate (*p*H 5·0) solution	102·0
Synthetic growth medium	45·0

Beer yeast cultured in a synthetic medium for 72 hr. at 30° C. was incubated in 10 ml. of medium containing 2 per cent glucose for 3 hr. at 30° C. The released nucleotide-phosphorus was determined as follows. The extinctions in the supernatant of the incubated media were measured at 240, 260 (E_{260}) and 280 mμ respectively, with a Beckman *DU* spectrophotometer, and the $E_{mμ}$ due to the released nucleotides was obtained by subtracting the blank (the medium free from cells) from the $E_{mμ}$ of the supernatant. Thus, the nucleotide-phosphorus could be calculated according to Ogur and Rosen (ref. 12)

strongly suggest that citrate has a chelating effect which affects the release of nucleotides from the microbial cells.

On the basis of these results, it might be suggested that the presence of citrate or other chelating substances in a medium may remove calcium or magnesium ions from the cell surface, leading to destruction of ribonucleic acid and present in it, and thus to a release of nucleotide fragments. Several facts support this. For example, pancreatic ribonuclease is inhibited by the addition of magnesium or calcium ions[5], and a ribonucleic acid-rich fraction obtained from the cells of *E. coli* contains more magnesium than other fractions[6]. Further, ribonucleoprotein has apparent[7,8] or latent[9] ribonuclease activity.

On the other hand, it has often been observed that the degradation of ribonucleic acid and the release of ribonucleate from yeast cells takes place by autolysis[10,11]. The release of nucleotides under our experimental conditions may also be connected with cell autolysis. However, it seems reasonable to distinguish between the two, since the release in our case did not result in the death of the cells or even in degradation of the cell protein. In contrast, a considerable release of amino-acids occurred only when the yeast cells were incubated in the acetate buffer, which accelerated release of nucleotide only slightly.

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NEW ANTIMETABOLITES OF VITAMIN B_{12}

By G. M. TIMMIS and Dr. S. S. EPSTEIN

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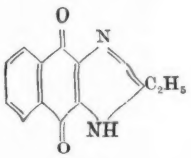
OUR interest in the role of vitamin B_{12} in cancer was first stimulated by the claims of Bodian[1], that prolonged regression of neuroblastoma in infancy was effected by massive dosage of the vitamin. It was therefore thought that the therapeutic effect claimed might be related to an imbalance in B_{12} metabolism caused by excess of the vitamin, and that such an effect might possibly be better produced by the administration of a B_{12} antimetabolite[2].

Hitherto, the only known B_{12} antimetabolites have been those described by Lester Smith[3], which were derived from the structure of B_{12} by very minor modifications, and also those which are relatively simple derivatives of the benzimidazole molecule[4,5]. The relation between tumour inhibition and anti-B_{12} action is shown in a member of the latter group by the effect of 2-ethyl-2,3-naphthimidazole-4,9-dione (I), which induced partial regression of a mouse mammary adenocarcinoma[6] and was also a competitive antagonist of vitamin B_{12} in an *E. coli* mutant[4].



(I)

We have explored a fairly wide variety of chemical types, chosen for their possible relation to B_{12} metabolism, for an antimetabolite action in a microbiological system. Our particular test organism was *E. gracilis* var. *z*—a highly sensitive B_{12}-dependent flagellated green alga. The basal growth medium and techniques of incubation were based on those described by Hutner *et al.*[7].

A series of approximately a hundred compounds was screened for inhibitory activity at 500 μgm./ml. levels, at low concentrations of vitamin B_{12}. Compounds producing more than 50 per cent inhibition of growth, as assessed in a colorimeter (measuring both turbidity and colour), were afterwards re-tested in definitive assays. An orthogonally designed test system was used, with varying concentrations of antagonist up to 500 μgm./ml. in the presence of 0, 10, 100 and 1,000 μμgm. B_{12}/ml. The growth response in these tests was measured by a pigment extraction technique using an acetone–ethyl acetate solvent system due to Epstein and Weiss[8].

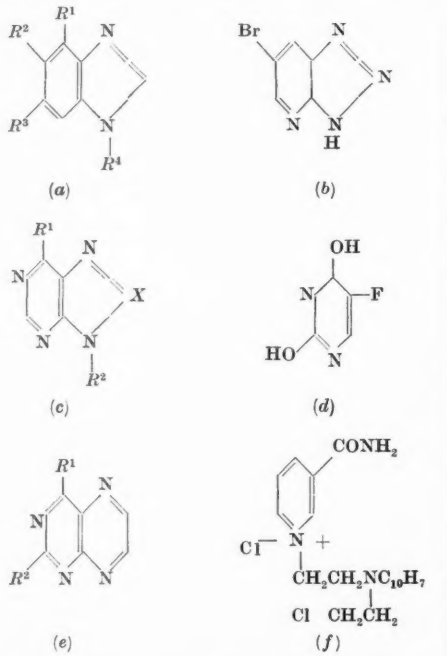
Results are expressed in terms of concentration of antagonist required to produce 50 per cent inhibition of growth at 10,100 and 1,000 μμgm. B_{12}/ml. respectively. The values given are only approximate. As can be seen from Table 1, seven out of the fifteen compounds so far found active on screening produced a competitive B_{12} antagonism.

We think that the discovery of an antimetabolite action in the pteridine and nicotinamide structures is interesting because, unlike the benzimidazoles or purines, they are not analogous in structure to any moiety of the B_{12} molecule. These observations indicate that there may be a variety of enzymes or co-factors closely concerned with the utilization of B_{12}, and that each one may be structurally specific for a particular inhibitor. Thus, the pteridines might compete with a pteridine type co-factor or metabolite for a particular enzyme, and the nicotinamide derivatives might interfere with some function of diphosphopyridine nucleotide (cf. Timmis[9]).

Table 1. Concentrations of Antagonists (μgm./ml.) producing 50 per cent Inhibition of Growth of *E. gracilis*
Results in the case of compounds producing competitive antagonism are given in italics

Structures of compounds tested, (*a*), (*b*) . . . , are on the right

Structural type	Formula	Concentration antagonist (μgm./ml.) required to produce 50 per cent inhibition of growth		
		B_{12} ($\mu\mu$gm./ml.) 10	100	1,000
(*a*) Benzimidazoles	4-nitro-6-chlorobenzimidazole ($R^1 = NO_2$, $R^2 = H$, $R^3 = Cl$, $R^4 = H$)	110	88	78
	5,6-dichlorobenzimidazole ($R^1 = R^4 = H$, $R^2 = R^3 = Cl$)	106	90	94
	4,5,6-trichlorobenzimidazole ($R^1 = R^2 = R^3 = Cl$, $R^4 = H$)	70	65	63
	4,5,6-trichlorobenzimidazole riboside ($R^1 = R^2 = R^3 = Cl$, R^4 = ribosyl)	*70*	*160*	*230*
(*b*) Diaza-benzimidazole	5-bromo-2,7-diazabenzimidazole	190	178	173
(*c*) Purines and azapurines	Purine ($R^1 = R^2 = H$, $X = CH$)	138	173	138
	Purine riboside ($R^1 = H$, R^2 = ribosyl, $X = CH$)	58	62	60
	6-mercaptopurine ($R^1 = SH$, $R^2 = H$, $X = CH$)	*200*	*>500*	*>500*
	9-furfuryl-8-aza adenine ($R^1 = NH_2$, R^2 = furfuryl, $X = N$)	*450*	*>500*	*>500*
	2-diethylamino-6-*p*-dimethylaminophenylethyl purine ($R^1 = (CH_3)_2C_6H_4N(CH_3)_2(p)$)	*150*	*>500*	*>500*
(*d*) Pyrimidine	5-fluorouracil	78	55	54
(*e*) Pteridines	4-mercaptopteridine ($R^1 = SH$, $R^2 = H$)	*95*	*350*	*333*
	2-amino-4-*p*-diethylaminostyrylpteridine ($R^1 = (CH)_2.C_6H_4.N(C_2H_5)_2(p)$, $R^2 = NH_2$)	*73*	*>500*	*>500*
	4-dimethylaminopteridine ($R^1 = N(CH_3)_2$, $R^2 = H$)	*400*	*>500*	*>500*
(*f*) Nicotinamide derivative	N-2-chloroethyl-β-naphthylaminoethyl-3-carbamoylpyridinium chloride	*200*	*430*	*415*



There are interesting differences between the results obtained in our microbiological system and in the *E. coli* mutant used by McNair Scott *et al.*[5]; for example, 6-chloro-4 nitrobenzimidazole was not a competitive antagonist in our hands, in contrast to 4,5,6-trichlorobenzimidazole riboside. On the other hand, McNair Scott *et al.*[5] found these compounds to be competitive and non-competitive antagonists respectively.

It is generally held that 6-mercaptopurine, one of the most effective drugs for the treatment of acute leukæmia, inhibits growth by interfering with nucleic acid and protein synthesis. Its action in this disease, however, has not with any certainty been related to an antipurine effect. Since we have found that this drug is a competitive B_{12} antagonist (Table 1), we would like to direct attention to the possibility that anti-B_{12} effect might be involved in the action of 6-mercaptopurine. This speculative suggestion could be supported by the view of Osgood[10] that the bone marrow in untreated pernicious anæmia shows several features in common with that in acute leukæmia. If the two diseases are accordingly related, acute leukæmia may, like pernicious anæmia, be dependent on some derangement of B_{12} metabolism, each in its own way.

Forrest *et al.*[11] have very recently isolated a simple pteridine from the bodies of blue-green algae. It is shown above that a number of pteridines of comparable simplicity are vitamin B_{12} antimetabolites in the B_{12}-dependent system *Euglena gracilis*. On reading our manuscript, Dr. G. E. Fogg suggested that the apparent inability of blue-green algae and Euglenidae to co-exist in Nature, a hitherto unexplained phenomenon, may be due to an antagonism between the blue-green algae pteridine and the vitamin B_{12} of B_{12}-requiring *Euglena*. We are now investigating this hypothesis.

A full report of this work will shortly be submitted to the *British Journal of Pharmacology and Chemotherapy*.

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LOW-TEMPERATURE AUTORADIOGRAPHY FOR THE DETECTION OF TRITIUM IN TISSUE, WITH REMOVAL OF LUMINESCENCE INDUCED BY TRITIUM

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NUMEROUS studies have shown inequalities in the distribution of labelled water in the different organs and various compartments containing liquid in the normal animal[1,2] during the pre-equilibrium period.

It seemed of interest to investigate by means of photographic recordings the kinetics of this distribution, working on animals (young rats) killed at fixed intervals of time. For that purpose we used the low-temperature autoradiographic technique[3,4], the only method which, because of the rapid freezing of the animal, eliminates post-mortem diffusion of the water while at the same time guaranteeing the absence of known pseudo-radiographic effects.

We wished first to obtain an image of homogeneous diffusion in the whole of the organism, under conditions close to equilibrium, and this formed the object of the present work.

The photographic recordings obtained by this technique, applied to young rats after administration of 20 mc. of tritiated water, gave unexpected results (Fig. 1*a*). They show no blackening corresponding to lungs, liver, spleen, muscle. On the other hand, a slight darkening shows up in the region of the intestinal walls and the subcutaneous tissue, and a very intense blackening at the sites of the eye, the brain, the preformed teeth, the stomach, the bladder and the intervertebral disks.

Since these results disagreed with the known distribution of water in mammals, we removed organs from young rats treated in the same way except that the dose injected was between 0·4 and 1·5 mc. of tritiated water (HTO) in a volume of 0·1–0·2 ml. of saline. The radioactivity of the water obtained by distillation of the organs was measured by liquid scintillation[5,6].

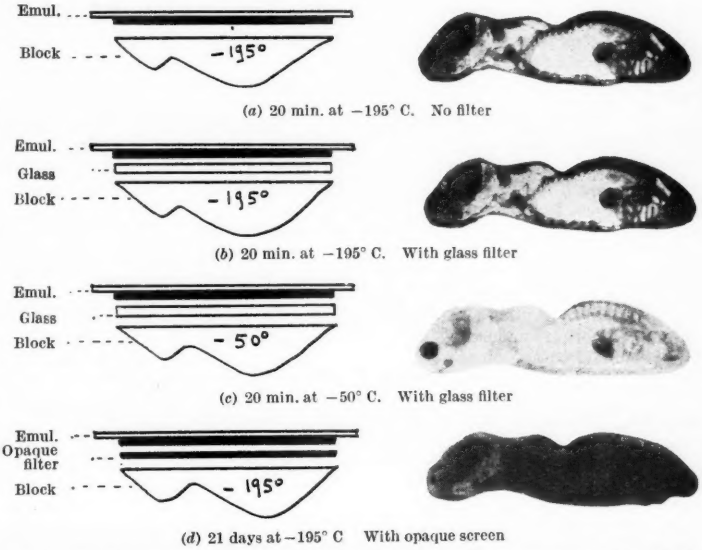
It is shown in Table 1 that the specific radioactivity of the water (mμc./mgm.) is of the same order for the blood and for organs such as the brain, the liver and spleen, the kidneys and the intestines. On the other hand, the milk in the stomach has not yet reached the isotopic concentration of HTO in the other organs. The specific radioactivity of the water in the total eye, the crystalline lens and the ocular residue after elimination of the crystalline lens is more or less identical with that of the blood. We have also noted that for the eye the radioactivity was entirely due to the water, and that no measurable tritium was incorporated in the dry residue.

Table 1. SPECIFIC RADIOACTIVITY OF WATER IN THE ORGANS OF YOUNG RATS WEIGHING 14–20 GM., AGED 6–14 DAYS, 10 MIN. AFTER SUBCUTANEOUS OR INTRAPERITONEAL INJECTION OF 0·1–0·2 ML. OF SALINE CONTAINING 0·4–1·5 MC. TRITIATED WATER; STANDARD DEVIATION ± 2 PER CENT

Organ	Millimicrocuries of HTO per mgm. water				
	Rat 21	Rat 23	Rat 15	Rat 19	Rat 20
Blood	—	44	88	80	80
Brain	44	43	90	92	75
Liver, spleen	45	—	—	88	90
Liver, spleen, kidneys	—	46	85	—	—
Bowel, small and large, with contents	48	45	80	92	91
Stomach containing milk	—	—	41	37	46
1 eye*	40	36	—	88	—
1 crystalline lens*	46	—	—	85	40
1 eye without crystalline lens*	42	—	—	90	—

* The water content is calculated on the basis of 70 per cent of water for the crystalline lens and 80 per cent for the rest of the eyeball.

In view of the disagreements found between the photographic recordings and direct measurements, we have looked into the mechanism of autoradiography itself in an attempt to find the cause of these paradoxical images; this has led to the discovery of a phenomenon of luminescence produced at low temperature under the influence of the very soft tritium beta-rays, actually within certain tissues of the organisms studied.



(*a*) 20 min. at −195° C. No filter

(*b*) 20 min. at −195° C. With glass filter

(*c*) 20 min. at −50° C. With glass filter

(*d*) 21 days at −195° C With opaque screen

Fig. 1. Two-day-old rat. Subcutaneous dorsal injection of 20 mc. deuterium oxide. 20 min. after injection

* Attachée de Recherche à l'Institut National d'Hygiene.

This is demonstrated by the following series of experiments (Fig. 1) carried out on rats 48-hr. old, killed 20 min. after dorsal-subcutaneous injection of 20 mc. of tritiated water.

(1) (See Fig. 1*a*.) The machined block at −195° C. is applied directly against the emulsion. The exposure lasts 20 min. at liquid-nitrogen temperature. In this way the paradoxical image described above is obtained.

(2) (See Fig. 1*b*.) Exposure of the same block at the same temperature for an equal period, but with a 1-mm. thick sheet of glass interposed, gives an image identical with the preceding one. Since, however, the maximum energy of tritium beta-particles (18 keV.) is not great enough to allow their passage through this thickness of glass, the image obtained is not due to a direct action of tritium radiation on the emulsion and can only result from a luminescence.

(3) (See Fig. 1*c*.) On repeating experiment *b* at a temperature of −50° C., a general decrease in blackening is noted, although the distribution is not altered.

(4) (Fig. 1*d*.) Finally, exposure for 21 days at −195° C. with a screen of opaque carbon paper interposed between the block and the emulsion gives a true autoradiographic recording on which quite a homogeneous distribution of blackening is observed.

From this limited study, some characteristics of this luminescence produced by the local absorption of tritium beta-radiation by certain tissues can be formulated. It becomes more intense as the temperature is lowered. For a uniform distribution of tritiated water in the organism, the intensity of this fluorescence varies greatly according to the nature of the tissue.

For the exposure time used, the threshold of blackening detectable on the emulsion for tritium beta-radiation is not reached at any point of the recording, while the phenomenon of luminescence reaches saturation in the region of the eye, as shown in Fig. 1*a*. When a screen opaque to light is interposed (Fig. 1*d*) the exposure time to obtain an appreciable blackening is 1,600 times longer than in the case of exposure without screen (Fig. 1*a*). It may seem surprising that part of the tritium beta-radiation (mean path in water, 1μ) can pass through a thickness of paper of the order of 50μ. We believe that the explanation may be found in the fibrous structure of the paper.

We may assume that the autoradiographic recordings obtained under the conditions of experiment *d* (with opaque screen) represent exactly the distribution of tritium at the surface of the machined block.

After dorsal subcutaneous injection of tritiated water, the autoradiographs show instantly homogeneous diffusion of the activity, the only exceptions being the bones, the teeth and the milk contained in the stomach. The teeth and the milk, however, form exceptionally luminescent zones. The region of dorsal injection naturally corresponds to a more intense blackening.

The detailed mechanism of this luminescence is difficult to interpret and belongs to the field of structural physics. We do not, in fact, know the chemical nature of the luminescent substances present in the frozen organs, still less their molecular structure at −195° C., or their behaviour in the presence of beta-radiation.

Validity of low-temperature autoradiography. The above statements could lead to some doubt concerning the value of recordings obtained by low-temperature autoradiography with radioelements other than tritium[3,4]. We have verified that this is not the case, in particular with sulphur-35 and calcium-45. Fig. 2*a* shows the result of autoradiography without screen carried out on a young rat, 90 min. after dorsal subcutaneous injection of 30 μc. of calcium-45 chloride, with a 17-hr. exposure. In Fig. 2*b* the interposition of a glass screen eliminates all recording for this exposure period (except for the left fore part of the head which was not covered by the glass sheet); the luminescence effect, therefore, does not appear under these conditions for calcium-45 radiation.

Fig. 2

The recording in Fig. 2*c* and *d*, carried out after interposition of a carbon paper screen and of an aluminium screen 0·2 mm. thick, respectively, can be strictly superimposed on the direct autoradiograph *a*, with the difference only of general blackening, which is slightly decreased by the screens used for *c* and *d*.

In tests carried out under the same conditions of metabolism and exposure after injection of 50 μc. of sulphur-35, by direct contact and with interposition of a carbon paper screen, an absence of luminescence was again observed.

We conclude that the luminescence effect of tissues at very low temperature is only detectable for extremely soft radiations such as that of tritium. It is advisable, however, in applying autoradiography at very low temperature to other radio elements, to verify that the interposition of an opaque filter does not modify the images obtained by direct contact between the block and the emulsion.

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A ZOOLOGICAL SURVEY OF MADAGASCAR

By Dr. RENAUD PAULIAN
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THE survey of tropical faunas has, for nearly two centuries, been largely limited to the haphazard methods of the general collector or to the painstaking but limited efforts of individual specialists. The results, as could be predicted, were of very doubtful value when either ecological or biogeographical projects were considered. However, they were extraordinarily successful in so far as they brought to light a very large number of new forms and built up our present knowledge of taxonomy. It unfortunately proved impossible to compare, with any accuracy, the faunas of two neighbouring areas for any scientific purpose. This did not prevent ambitious workers from building biogeographical or ecological systems on a very large scale. Yet to the man working in the field these systems seemed always rather unsafe, even though they called in all the resources of the geological and statistical methods and very strict logical constructions.

As a tropical country, Madagascar had for some time the very rare distinction that a series of monographs in the *Histoire Physique, naturelle et politique de Madagascar* had been published by the combined efforts of A. and G. Grandidier. It could thus compare with Central America, Seychelles, Hawaii or British India. But even these monographs, mainly published during the second half of last century, are now considered out of date.

In the years following the Second World War, research work on tropical faunas has started on completely revolutionary lines. New research institutions and groups are either working permanently in tropical areas or covering these in terms of long-range work. They are thus enabled to collect intensively the whole year round, using breeding methods and many new collecting systems. Such teams are at work in the Belgian Congo, Micronesia, Melanesia, etc.

The Zoological Survey started in Madagascar in 1947 and is still at work. It would seem from published results[1] that it has been particularly successful. It is centred on the Zoology Department of the Institut Scientifique de Madagascar, an institution covering pure and applied research in most fields of human, animal and plant biology and in soil science, hydrology and geography. The Institute itself belongs to a chain of tropical institutes created after 1946 by the French Office de la Recherche Scientifique et Technique Outre-Mer in what was then the French Union and is now the French Community.

It may be of interest, considering the variety of results obtained in the short period 1947–59, to give some details on the practical organization of the Survey before describing the changes wrought in our knowledge of the local fauna.

Considering that only limited means could be brought to bear on the problem of such a survey, the following principles have been applied.

(*a*) The formation of a mobile team with one or two European scientists, a number (3–6) of native assistants, a 'Landrover', and all the basic collecting equipment. This team has the use of a small permanent workshop in the centre at Tananarive for the building of new gadgets, and the constant upkeep of the material used.

(*b*) A central office, with one to three European scientists and three or four native assistants, which receives the collections, prints the labels, mounts and sorts the material and makes a preliminary study of all collections. More breeding work is carried on in the insectarium of the Central Office.

(*c*) A very large team of honorary research workers, specialists of international reputation, from practically every country of the world. They have agreed to work out collections made in Madagascar from their special groups. They are responsible for describing any new or remarkable forms and preparing revisions of such families, tribes or genera which may be considered as fairly well known. More than a hundred specialists have been enlisted in this way.

(*d*) A practical organization enlisting the co-operation of well-known foreign specialists, giving them all possible help and practical local guidance, helping them with the necessary camping and collecting outfit, etc. This organization can help specialists who come to Madagascar on their own, arrange for joint expeditions, or even finance complete expeditions, to which they are invited. In this way, during the past ten years, no less than thirty-three zoologists have taken part in the survey and some of them have spent more than a year in Madagascar or have come several times. The 'geographical distribution' of these specialists is worth noting: two Mauritians, five South Africans, one Rhodesian, two East Africans, three Swiss, three Austrians, three Italians, one German, eight Americans, one Australian and five French. In all cases the material collected or the observations made are embodied in the general publications of the Institute.

(*e*) Means for regular and so far as possible prompt publication of results. These have been obtained by the channel of two new scientific periodicals: papers on the local fauna make up the whole of the *Memoires de l'Institut Scientifique de Madagascar*, Series A (zoology) and E (entomology), and are the main topic in the *Naturaliste malgache*. Whenever a group seemed sufficiently well known, a revision is published in the series "Faune de Madagascar", on the plan of the well-known "Faune de France". Apart from the nine published volumes of this series, three other volumes are ready to be printed and half a dozen others (covering such groups as birds, Acridoidea, Vespidae, Tingidae) are being actively prepared.

It was planned at the start to limit the survey to Madagascar proper. However, it was soon found necessary to cover the insular environment, the Mascarenes, the Comoros and the small atolls or coral reefs (Tromelin, Europa, Glorioso). Although the knowledge we may gain of their fauna is completely independent of Madagascar proper, it is a great help towards an understanding of the malagasy fauna itself.

It is still too early to give a complete analysis of the results of the Survey. More than a million specimens have been collected, covering the whole animal kingdom from protozoa to mammals, and a very large part of this material is still undergoing systematic study.

From the purely taxonomical side more than 2,500 new forms (subfamilies, tribes, genera and species) have been described. Many orders have been discovered on Madagascar proper (for example, freshwater Coelenterata, Porifera, Bryozoa; in Insecta: Zoraptera, Plecoptera, Diploura, Protoura; in Chelicerata: Palpigrada, Uropyga, Amblypyga; in Crustacea: Syncarida) and many more groups. No animal group has remained untouched, and often the known number of forms has risen by as much as 500 per cent.

The very great variety of new forms found in the island has completely changed its aspect and helped to build an entirely new system of biogeographical relations.

The Survey has opened up a number of new biota: many new groups of cave-dwelling forms have been detected, such as scorpions and Homoptera, that were not known previously from this habitat; a very large and highly specialized blind soil-fauna, probably one of the richest in the world; a series of crustaceans and worms inhabiting interstitial waters, both salt and fresh; an original intertidal fauna of land arthropods; a rich fauna living in the water found in leaf axils of palms, Musaceae, Aroideae and in bamboos. This last fauna is exceptionally large and its members range from frogs to insects and crustaceans; mosquitoes play a very important part, with high speciation and remarkable morphological adaptations.

Less original, but quite as important results have been obtained by the systematic breeding of parasites (chalcidoids, tachinids, etc.) and of leaf-mining and gall-making insects. These researches have brought to light new biological facts for known groups, such for example as the existence of leaf-mining drosophilids.

In the extreme north of Madagascar, where recent volcanoes with rain forest co-exist with deciduous forest on limestone, sandstone or lateritic soils, a comparative study of the fauna has been most rewarding. Not only does it give many remarkable new forms but also helps in the understanding of factors underlying speciation, which is one of the most remarkable facts in the local fauna.

Some facts have been established on a firm basis and allow for biological discussion. For example, the remarkably active speciation referred to, with a high number of sympatric species, the numerous missing elements in the fauna as compared with continental Africa or the Indian subcontinent; the place and significance of the austral element; the wide plasticity of most new imported forms, etc. A number of principles underlying the building up of the fauna on small isolated islands have also been found. All these have shown that Madagascar was one of the most remarkable natural laboratories in the world for the study of species formation, adaptation and evolution. A considerable research effort should be started along such lines in the near future.

By necessity, the work until now has been mostly taxonomical; ecology has only been considered in so far as it opened up new biota; biological research has been limited to breeding work. But with the progress of the general catalogue of Madagascar's fauna research work on biology, ecology and physiology will soon be possible. If the method used has delayed a biological approach to Madagascar, it is believed that it has ensured a sure and efficient basis for such an approach and has saved many efforts and many errors due to an insufficient basic knowledge of the local fauna. It has in this way proved time-saving. The results of this Survey have shown also how completely misleading was the previous knowledge of a tropical fauna which one considered usually as fairly well known.

Thus it is quite evident that the Zoological Survey will never be finished, just as it is not finished in Britain or in France, but it may be brought to such a stage that, for most practical purposes, it can be considered as sufficient.

[1] *Mémoires de l'Institut Scientifique de Madagascar*, série A, 1–12; série E, 1–12. *Naturaliste malgache*, 1–10. *Faune de Madagascar*, 1–9.

BIONOMICS OF THE BRUSH-TAILED OPOSSUM *TRICHOSURUS VULPECULA* IN NEW ZEALAND

By R. I. KEAN

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DURING the latter part of the nineteenth century five Australian marsupial species were established in New Zealand—four wallabies, *Protemnodon rufogrisea*, *P. eugenii*, *P. bicolor* and *Petrogale penicillata*, together with one opossum, *Trichosurus vulpecula*.

Of the wallabies, only the first two cause loss to agricultural production, and that is not yet great because these animals occur only in restricted areas; but the opossum, which has some claim to being considered the most successful exotic mammal, is widespread, with an ecological range extending from suburban areas, through farm lands, to all types of forest. The significance of this animal to agriculture has not been thoroughly investigated; but in timber production serious loss has been caused through induced malformation of trees, mainly *Pinus* species, and in protection forests destruction of trees has been spectacular.

Economic need led to the commencement, in 1953, of systematic research on the opossum, and, to a lesser extent, on the wallabies.

Measures for control of the opossum will have to lean heavily upon a sound understanding of bionomics; but, since ecological steps are slow and are not well understood by the public, the more efficient use of poisons has been investigated, particularly methods involving application by aircraft.

Some opossum populations in New Zealand maintain balance with little artificial control; in these a female first rears an offspring in her third year, the kitten being ready for independent existence when the mother is 2 years 7 months old. A single kitten is reared in each succeeding year until about the tenth year. The bearing female exhibits little interest in the small young, but during the first two months of her kitten's pouch-free life she shows strong maternal attachment, and is aggressive towards other opossums.

Under certain conditions (probably low population pressure, firmly established territory, and abundant food) some females rear their first young during their second year instead of in the third, and also rear two young annually.

The female reproductive system is basically similar to its eutherian counterpart, both in that the vagina remains closed until first required for reproduction, and in that it is seasonally occluded thereafter. The divergence from the eutherian lies mainly in the extreme heterochrony of development—the retention by the adult female of an embryonic tissue, the urogenital strand[2]—and in the relative lack of structure of the caudal portion of the vagina. Both these marsupial characters appear to be simplifications permitted by the extreme specialization of the embryo, namely, its small size and its faculty for active locomotion before birth. Seminal ducts, the so-called lateral canals, appear to have arisen phylogenetically, as suggested by T. H. Huxley[3], at some late prototherian stage of organization. The opening of the median vaginal canal and the closing of the lateral canals during late pregnancy, apparently in response to hormones, support Huxley's hypothesis[4].

Œstrus is marked by desquamation of semi-cornified cells from the urogenital sinus. The cycle, measured partly by smears and partly by direct observation, has been recorded by the writer as 22 or sometimes 23 days. This is less than the 24½ day average (21–30) of Lyne[1] derived from smears that were made daily.

Coition occurs on the night following œstrus (if œstrus is defined as the diel period of restlessness) and the single ovum is shed spontaneously approximately 24 hr. later.

The period of gestation was found to vary, apparently because of disturbance to the female. Two pregnancies, timed from observed copulations, were completed in 17 and 18 days ; others were lengthened, possibly to as long as 40 days when smears were taken, but this technique was usually associated with infertility. One female from which smears were taken at two-day intervals was killed 28 days after external signs of pregnancy became obvious. Copulation had occurred (ascertained by means of smear) 7 days before death. The left ovary showed a 4·5-mm. corpus luteum associated with a 4·8-mm. blastocyst, and the right ovary an involuted 2·6-mm. corpus luteum associated with a 3·4-mm. blastocyst. In this case, superfœtation appeared to have occurred in the course of a prolonged gestation.

Neither post-partum œstrus nor delayed implantation, as found by Sharman[5] in the wallaby *Setonix*, and almost certainly by Bowley[6] in the primitive phalanger *Cercartetus*, occurs in *Trichosurus*. Sharman found that stimulation of the pouch by a contained young inhibited development of the post-partum blastocyst, but the present work on *Trichosurus* suggests that generalized stress applied to a female in early pregnancy resembles, in its effect upon the blastocyst, the maternal response to pouch young.

Sperm have been found in the urogenital smear only from the third to fifth day post-coitus. This result appears to be due to aspermous seminal fluid of the final ejaculation which, within about one minute, sets to form firm white copulatory plugs that completely fill the lumina of the lateral canals. The plugs are later absorbed *in situ*. Muscular occlusion and epithelial proliferation again seal the caudal ends of the lateral canals during early pregnancy.

Male fertility, contrary to the findings of Bolliger[7] on captive opossums, shows endogenous fluctuations which may be cyclic. The incidence of sperm is lowest during summer, when probably most males are aspermous for an overlapping two-month period, but, in addition to this, some males in all age classes are aspermous in each month of the year. Regression of testis and urethral bulb occur, but these are not completely synchronous with the fluctuations of the cauda epididymis and its sperm.

Reproduction is highly efficient. Of the 106 adult females which were caught (excluding 15 pregnant or anœstrous ones) 90 carried pouch young and single corpora lutea on the ovaries, indicating almost certainly that for 84·9 per cent of this sample the first œstrus of the season had been fertile, and that the resultant young had been successfully established. In the case described by Lyne[1] the newly born young's initial fall and its subsequent failure to take the teat might have been attributable to a defective maternal behaviour pattern induced by captivity.

The growth of the young resembles that of man (on an equivalent time-scale) rather than that of a typical mammal ; a long period of slow growth is broken by an intense adolescent spurt, which is followed by a further slow period which continues until maturity. The opossum in its adolescent growth is less adaptable than man, who can defer the spurt when subject to stress ; although the opossum takes three years to reach full maturity, rapid growth can occur only during the one restricted period[8].

The demands of the small young upon its parent are negligible during the first four months of life, and the bearing female passes through the period of winter stress in better condition than the male. The young makes its main growth in the spring, but slows down during the autumn period of maximum abundance, accumulating some fat before winter.

Predation upon the forest opossum in New Zealand is applied only by man. Natural regulation of numbers occurs through the low productivity. The birth-rate can be greatly accelerated, when conditions are favourable, by the survival of young from the usually unproductive second-year juveniles, and by a spring breeding season supplementing the normal autumn one.

An ultimate safeguard to forests, which are the natural habitat of the opossum, lies in the stenophagy that is characteristic of the Phalangeridae. Only a limited number of tree species are killed (except by starving opossums).

Present forest destruction has two basic causes : first, in the previous absence of herbivorous mammals, palatable tree species have grown in vulnerable compact stands which could not develop to-day ; and secondly, euryphagous deer and goats, by the destruction of undergrowth, have both prevented forest regeneration and intensified opossum pressure upon the mature trees.

These investigations are continuing, and it is intended that full reports will be published later.

[1] Lyne, A. G., Pilton, P. E., and Sharman, G. B., *Nature*, **183**, 622 (1959).
[2] Hill, J. P., *Proc. Linn. Soc. N.S.W.*, **24**, 42 (1899).
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[8] Kean, R. I., "Natural Control in Populations of the Opossum *Trichosurus vulpecula* in New Zealand" (unpublished).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, December 7

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. B. Jacobsen: "Frequency Patterns for Multiple-Radio-Channel Routes".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. Max Derruau (University of Clermont-Ferrand): First of three lectures on the "Central Massif of France".* (Further lectures on December 8 and 10.)

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 5.30 p.m.—Dr. M. Eigen (Max-Planck-Institut für Physikalische Chemie, Göttingen): "Relaxation Spectra of Fast Chemical Changes in Solution".*

MANCHESTER LITERARY AND PHILOSOPHICAL SOCIETY (in the Reynolds Hall, Manchester College of Science and Technology, Sackville Street, Manchester), at 5.45 p.m.—Dr. J. Needham, F.R.S.: "Hydraulic Engineering and Society in Ancient and Medieval China" (Wilde Memorial Lecture).

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. G. Pask: "Discontinuous Skill Teaching Machines".

Tuesday, December 8

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Dr. T. H. Rose and Mr. P. H. Brown: "Experiments Comparing a Number of Conditions for the Improvement of Glasshouse Soils"; Mr. J. Webber and Mr. F. W. Shepherd: "Experiments Comparing Bulky Organic Manures for Horticultural Crops"; Dr. F. Haworth: "Fertilizer Responses of some Vegetable Crops".

INSTITUTE OF MARINE ENGINEERS (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. A. Lindén: "Recent Development of the Gotaverken Engine".

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 5.30 p.m.—Dr. M. Eigen (Max-Planck-Institut für Physikalische Chemie, Göttingen): "Proton-conducting H-bond Systems and their Analogies to Electronic Semiconductors".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. G. Lajtha: "Autoradiography in Bone Marrow Studies". (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.* (Further lecture on December 10.)

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. E. K. Armstrong and Mr. R. E. Stevenson: "Some Practical Aspects of Compressor Blade Vibration".

Wednesday, December 9

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. J. D. N. Nabarro: "Laboratory Investigations in Endocrinology".*

INSTITUTE OF PHYSICS (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Sir Edward C. Bullard, F.R.S.: "Geological Time".

SOCIETY FOR PSYCHICAL RESEARCH (at Leighton House, 12 Holland Park Road, London, W.14), at 6.30 p.m.—Dr. Letitia Fairfield: "Children and Witchcraft".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. S. H. Bell: "Electron Microscopy and Paint Technology".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Annual General Meeting, followed by Dr. H. J. Wallis, Mr. S. S. Kind and Dr. A. S. Curry: "Biological Methods in Forensic Science".

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Dr. Elizabeth Laverick: "Radar and Telecommunications Research and Development".

Wednesday, December 9—Friday, December 11

CROP PROTECTION AND PEST CONTROL EXHIBITION (organized by *World Crops*, at the Seymour Hall, Seymour Place, London, W.1).

Thursday, December 10

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. B. Corbyn and Mr. N. L. Potter: "The Characteristics and Protection of Semiconductor Rectifiers".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Sheila T. Callender; "Iron Absorption".* (Last of the fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Thursday, December 10—Friday, December 11

BRITISH INSTITUTE OF RADIOLOGY, incorporated with the RÖNTGEN SOCIETY (at Church House, Westminster, London, S.W.1)—Annual Congress and Exhibition.

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 9.30 a.m. daily—Discussion on "The Biology of the Southern Cold Temperate Zone" opened by Prof. C. F. A. Pantin, F.R.S.

Friday, December 11

ROYAL AERONAUTICAL SOCIETY (at the Institution of Mechanical Engineers, Birdcage Walk, Westminster, London, S.W.1).—Symposium on "Flight Safety" (All Day Discussion).

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Prof. A. Haddow, F.R.S.: "The Present and Future Relationship of Cancer Research to Medicine".

INSTITUTE OF PHYSICS (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. F. Sayers: "The Ultrasonic Camera—an Alternative Approach to Ultrasonic Testing".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Sir Willis Jackson, F.R.S.: "The Trends of Electrical Progress and Their Implications".

ROYAL AERONAUTICAL SOCIETY, AGRICULTURAL AVIATION GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. Peter King: "Aerial Operations Planning in the United Kingdom—Results and Difficulties".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. E. D. Bishop: "The Menace of Vibration in Engineering".

Saturday, December 12

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Philip Hunter-Jones: "The Desert Locust".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

TECHNICAL OFFICER (with a university degree or comparable qualification in physics or electrical engineering or other approved subjects) IN THE DEPARTMENT OF PHYSICS—Dr. F. B. Kipping, University Chemical Laboratory, Lensfield Road, Cambridge (December 2).

PRINCIPAL SCIENTIFIC OFFICER (with research experience, preferably, but not essentially, in the chemistry of fats or related substances) IN THE FATS RESEARCH LABORATORY of the Department of Scientific and Industrial Research, New Zealand, to undertake investigations on the composition and significance of fats and related substances, including phospholipids and sterols present in animal and plant tissues—The High Commissioner for New Zealand, New Zealand House, 415 Strand, London, W.C.2, quoting Ref. No. B.11/21/22 and mentioning *Nature* (December 4).

ASSISTANT OR ASSOCIATE PROFESSOR OF APPLIED MATHEMATICS—The Chairman, Department of Mathematics, McMaster University, Hamilton, Canada (December 10).

LECTURER OR ASSISTANT LECTURER (with at least a good honours degree in physics, and preferably experience in teaching and/or research) IN PHYSICS at the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (December 15).

LECTURER/SENIOR LECTURER (with an honours degree in chemical engineering and some teaching or industrial experience) IN CHEMICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, December 19).

SENIOR LECTURER or LECTURER IN PATHOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square London, W.C.1 (December 22).

SENIOR TECHNICIAN or TECHNICIAN IN THE DEPARTMENT OF BACTERIOLOGY, and a SENIOR TECHNICIAN or TECHNICIAN IN THE DEPARTMENT OF SURGERY, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 22).

ENTOMOLOGIST, Grade C (with a good honours degree in entomology (zoology) and a particular interest in biological control work)—The Director, Institute of Biological Control, Science Building, Carling Avenue, Ottawa, Ontario, Canada (December 31).

HEAD (with a degree in electrical engineering or Corporate Member of the Institution of Electrical Engineers) OF THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Principal, Coventry Technical College, Butts, Coventry (December 31).

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LETTERS TO THE EDITORS

ASTROPHYSICS

Correlation between the Intensity of the Umbra of Sunspots and Enhanced Radiation on 200 Mc./s.

ENHANCED solar radiation at metre wave-lengths is generally supposed to originate within a restricted area of the corona above a sunspot. The position of the radio source indicates that the existence of a sunspot is a necessary condition for the emission of excess radiation.

Only a few sunspots, however, are associated with noise storms, and the question arises what makes certain sunspots show noise activity. If characteristic features of noise-active sunspots could be found, further information about the generation mechanism might conceivably be deduced. Observable properties, like area, life-time and magnetic field-strength of sunspots have been tested for correlation with noise activity, but the correlation is low.

At the Solar Observatory, Harestua, the relative intensity of umbra and penumbra of sunspots has been continuously observed since April 1959 with the aid of a pinhole photometer, operating on photoelectric principles. Measurements are made in three different wave-length regions: centred on λ4250, λ5500 and λ8500. Radiometer and interferometer measurements of enhanced radiation on 200 Mc./s. have been made during the same period. We are thus able to determine the degree of correlation between intensity of the umbra and the noise activity of sunspots.

Measurements of intensity of the umbra have been corrected to a first approximation for the light scattered from penumbra and photosphere, following the method described by Korn[1]. These corrections are usually small, as we have only considered spots with areas larger than about 400 millionths of the solar hemisphere. When more spots than one have been measured in a group, we have used the intensity of the umbra of the largest spot in the analysis.

In Fig. 1 the relative intensity of the umbra $I\mu/I_{ph}(\lambda_{5500})$ of a spot is plotted against the daily mean flux density on 200 Mc./s. received from a source situated above the spot. Observations where it is uncertain whether the spot in question is responsible for the noise activity are also included.

Fig. 1 shows that there is a high correlation between darkness of the umbra and noise activity of sunspots. The diagram indicates that in order to show noise activity, the intensity of the umbra must be smaller than a threshold value (about 0·20 at λ5500). The strongest radio sources tend to be associated with rather dark spots. The same results are obtained in the other wave-length regions (λ4250 and λ7500).

Only a slight correlation exists between noise activity and area of the sunspot for areas exceeding 400 millionths of the solar hemisphere. Nor does the intensity of the umbra appear to be determined by the area alone. This indicates that the results shown in Fig. 1 cannot be explained as a consequence of a decrease in intensity of the umbra and a rise in noise activity with increasing magnitude of the spot.

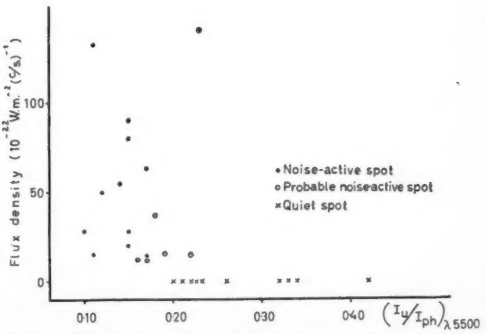

Fig. 1. Relative intensity of umbra of sunspot plotted against the daily mean flux density on 200 Mc./s. for the same spot

Although more definite conclusions can not be drawn at present, the observations single out the relative intensity of the umbra as a characteristic feature which may provide information about the mechanism of noise generation.

I wish to thank Mr. P. Ofstad for making available the observations with the pinhole photometer.

PER MALTBY

Solar Observatory,
Institute of Theoretical Astrophysics,
University of Oslo.
Sept. 19.

[1] Korn, J., *Astro. Nachr.*, **270**, 105 (1940).

RADIOPHYSICS

Spaced Observations of Radio Noise from the Outer Atmosphere

IT has recently been suggested[1] that during some types of radio noise storms generated in the Earth's outer atmosphere the source of the noise may remain almost constant in position in Right Ascension. If this were so, it would be expected that the arrival of the storm would be recorded at almost the same local time at places of different longitude, rather than simultaneously. To test this idea and more generally to investigate the extent to which observations of the noise are correlated at different places, recordings have been made during June and July 1959, at Camden N.S.W. (Latitude 34°03′S Longitude 150°42′E) and Adelaide (Latitude 34°56′S Longitude 138°53′E). The geomagnetic latitudes are 42°S and 45°S respectively. The noise level in a frequency band 1 kc./s. wide centred at 4·5 kc./s. was recorded using conventional techniques.

It was found that, in the majority of cases, noise bursts were recorded simultaneously at Camden and Adelaide. Of 18 bursts only 5 were recorded at Adelaide without being recorded at Camden and only 1 at Camden but not Adelaide. Figure 1(*a*) shows a typical example of a record with good detailed agreement in the amplitude variations at the two places. 8 were of this type.

On 4 occasions bursts were recorded, where although the minute to minute variations were similar,

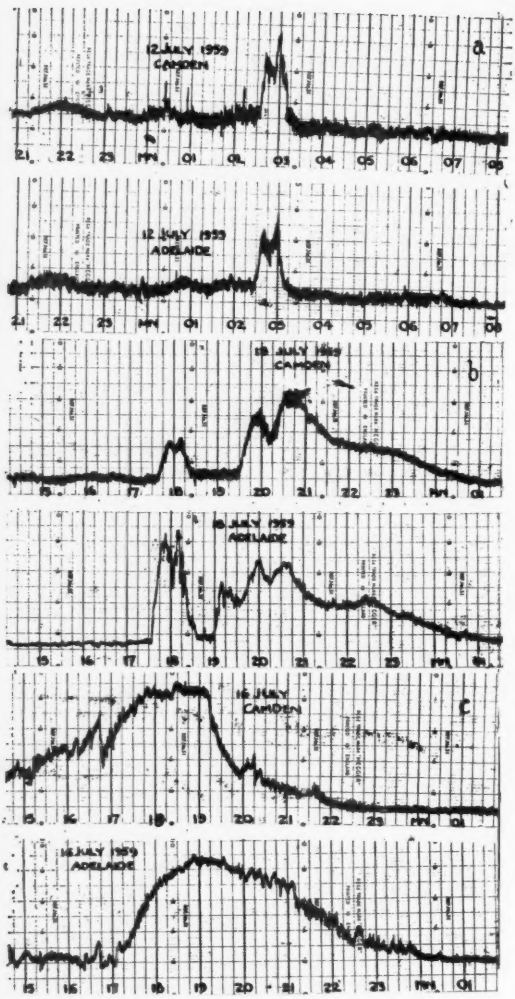

Fig. 1. Sample records of 4·5 kc./s. noise bursts observed at Camden and Adelaide. Ordinates, relative amplitudes ; abscissæ, Eastern Australian Time (hr.).

there was a difference in the trend of the peak amplitudes. An example is shown in Figure 1(*b*). On this and 3 other occasions the peak amplitude increased at Camden while decreasing at Adelaide. On 1 occasion the reverse occurred.

The only record showing a distinct time delay between the noise amplitude at Camden and Adelaide was obtained during the major geomagnetic storm and aurora of July 15, 1959, the only such occurrence during the present series of observations. This record is shown in Figure 1(*c*). The first part of the burst occurred very nearly at the same local time at both places, the difference in universal time being 41 minutes compared with a local time difference of 48 minutes.

From these observations it appears that low-frequency radio noise bursts may be detected over regions of the earth considerably greater in extent than the 1,000 km. separating the two recording stations. The simultaneity of the amplitude variations on most occasions implies that these regions are normally stationary with respect to the Earth. Nevertheless, the record of July 15 shows that this is not always so, although more examples are needed to establish storm patterns with delays equal to local time differences.

G. R. A. Ellis
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Upper Atmosphere Section,
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Industrial Research Organization,
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J. R. V. Groves

Weapons Research Establishment,
Salisbury, South Australia.

September 15

[1] Ellis, G. R. A., 'Planetary and Space Science' (in the press).

CHEMISTRY

Diffusion of Exchangeable Cations in Vermiculite

Vermiculite consists essentially of mica-like silicate layers separated by double sheets of water molecules carrying exchangeable cations, the cations being located midway between the water sheets in octahedral co-ordination[1]. In certain instances, replacement of one type of interlayer cation by another causes swelling or shrinking of the crystal lattice perpendicular to the plane of the silicate layers. These changes in the *c*-dimension of the unit cell are readily observable by X-ray diffraction procedures ; replacement of magnesium by strontium for example, involves an increase in interlayer distance of about 0·6A., corresponding to an increase in basal spacing from 14·4A. to 15·0A. When a flake of vermiculite in which interlayer magnesium has been partly replaced by strontium is examined by X-rays, 14·4A. and 15·0A. reflexions are observed together, with the latter growing in intensity at the expense of the former as the replacement proceeds (Fig. 1).

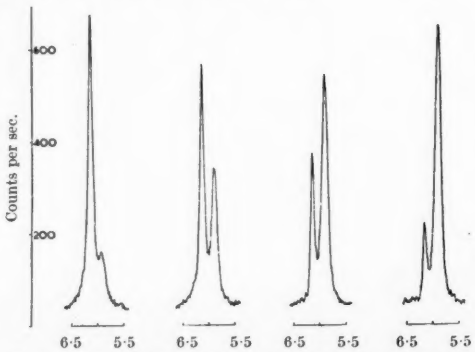
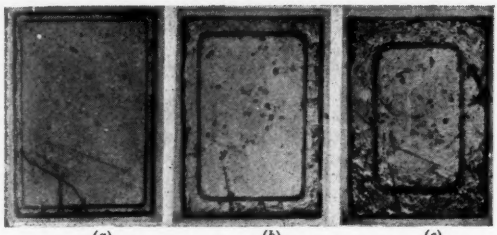


Fig. 1. X-ray diffractometer traces showing progressive changes in the basal reflexions of vermiculite during the replacement of interlayer magnesium by strontium (magnesium-lattice d_{001} = 6·2°2θ = 14·4A. ; Sr-lattice d_{001} = 5·9°2θ = 15·0A.).

Although measurements of this kind provide limited information regarding rates of exchange, they acquire greater significance when used in conjunction with the simple optical technique described below, which is basically similar to that described by me some years ago for measuring the rate of entry of water molecules into partially dehydrated vermiculite crystals[2]. As in the case of water adsorption, the expansion of the lattice which accompanies the entry



(a) (b) (c)

Fig. 2. Photomicrographs taken in transmitted light at intervals during the replacement of interlayer magnesium by strontium in a vermiculite flake (approx. dimensions, 1·5 mm. × 1·0 mm. × 0·1 mm.).

of the exchanging ions proceeds in a regular fashion from the edges towards the centre of a flake. The effect can be observed in transmitted light using low-power microscopy, provided an iris diaphragm (stopped down) is placed behind the objective. The flakes are cleaved so as to be about 0·1 mm. thick and cut to lateral dimensions of 1 or 2 mm. Under these conditions, the boundary between the 14·4A. 'magnesium-lattice' and the 15·0A. 'strontium-lattice' is seen as a dark line parallel to the flake edges, and representing a sharp increase in thickness of about 4 per cent between the central and external portions of the flake (Fig. 2). In a flake free from cross-cracks, the movement of the boundary during exchange is isotropic within the plane of the silicate layers.

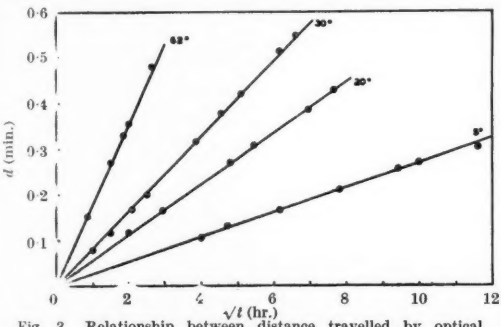
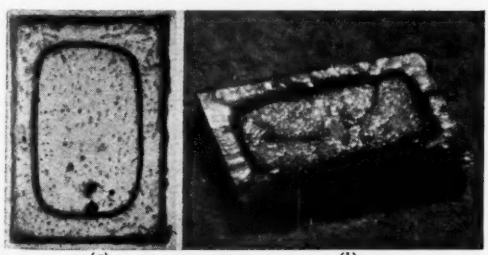


Fig. 3. Relationship between distance travelled by optical boundary and time of treatment during the replacement of interlayer magnesium by strontium in vermiculite at various temperatures (deg. C.).

When a flake of magnesium-vermiculite is immersed in 2*M* strontium chloride solution, the rate of movement of the boundary is found to be proportional to the square root of the time of treatment, indicating a diffusion-controlled process (Fig. 3). Measurements at various temperatures show that the reaction has an apparent activation energy of 12k. cal. per gm.-ion, which is several times greater than the energies involved in the diffusion of ions in aqueous solutions. Studies of the exchange diffusion of various inorganic cations in vermiculite are currently in progress in this laboratory, and further details will be published at a later stage.



(a) (b)

Fig. 4. Photomicrographs of vermiculite flakes in which interlayer magnesium has been partly replaced by *a*, butylammonium and *b*, cetylpyridinium ions (*a*, in transmitted light; *b*, in reflected light).

It may be of interest to record that the methods described above are also applicable to the study of the exchange of inorganic by organic (or organic by organic) cations. Fig. 4 illustrates the appearance of the 'exchange boundaries' in flakes in which partial replacement of interlayer magnesium by butylammonium and cetylpyridinium ions has taken place, the swelling of the crystal lattice being about 25 per cent and 150 per cent respectively.

G. F. Walker

Chemical Research Laboratories,
Commonwealth Scientific and
Industrial Research Organization,
Melbourne.
July 10.

[1] Mathieson, A. McL., and Walker, G. F., *Amer. Min.*, **39**, 231 (1954); Mathieson, A. McL., *Amer. Min.*, **43**, 216 (1958).
[2] Walker, G. F., *Nature*, **177**, 239 (1956).

BIOCHEMISTRY

The N-Terminal Amino-acids of α-Casein

As a result of previous work[1-3], it has been concluded that there exist in α-casein, arginine and lysine residues carrying free α-amino groups and occupying N-terminal positions in open polypeptide chains. Estimates of the amounts of such arginine and lysine have however varied within wide limits. Furthermore, the α-casein employed in two at least of these investigations[1,2] was prepared using a fractionation procedure now known to yield α-casein contaminated with k-casein.

End-group analyses have now been performed on preparations of α-casein obtained from skimmed milk by a modification of the procedure of Waugh and von Hippel[4]. α-casein prepared in this way was electrophoretically homogeneous, and was free from k-casein as judged by its stability towards rennin. The determination of the terminal amino-acids was made by way of dinitrophenylated α-casein following the principles of Sanger[5]. α-Casein was dinitrophenylated with 1-fluoro-2:4-dinitrobenzene in aqueous solution at *p*H 8 and 40°C. Carbon dioxide was excluded and the *p*H maintained at 8 throughout, by addition of standard alkali. Measurements of the light absorption at 355 mμ of this dinitrophenylated α-casein indicated that substitution by 55 dinitrophenyl residues per 10^5 gm. protein had occurred, compared with the value of 45 reported by Wissmann and Nitschmann[2].

Liberation of the dinitrophenyl amino-acids was effected by heating the dinitrophenylated α-casein in 5·7*N* hydrochloric acid for 16 hr. at 110°C. The ether-soluble material from the hydrolysis mixture was examined using a two-dimensional chromatographic procedure which was essentially that described by Levy[6]. The sole dinitrophenyl amino-acid present was identified, first by its position on the chromatogram, and secondly by direct comparison on the same sheet of paper with an authentic sample, as α:ε-didinitrophenyl lysine. Further confirmation of identity was obtained by its conversion to lysine on heating with concentrated ammonia in a sealed tube at 100°C. For quantitative determination, the didinitrophenyl lysine was eluted from the chromatogram with 1 per cent sodium bicarbonate solution, and

the light absorption at 355 mμ of the resulting solution measured. Recovery experiments with authentic α:ε-didinitrophenyl lysine showed that only 20 per cent remained unchanged after the hydrolytic and chromatographic procedures. Allowing for this, the N-terminal lysine in α-casein amounted to 0·3 mole per 10^5 gm. protein, which is approximately 20 per cent of the values reported by Wissmann and Nitschmann[2], and by Mellon *et al.*[1], and only about 4 per cent of that found by Ise[3].

The aqueous solution remaining after ether-extraction of the hydrolysis mixture was examined by chromatography on paper using the *tert.*-amyl alcohol system of Blackburn and Lowther[7]. Apart from ε-dinitrophenyl lysine arising from non-terminal lysine, the only dinitrophenyl amino-acid present was dinitrophenyl arginine. In addition to identification by direct comparison with authentic samples, the dinitrophenyl arginine was characterised by its positive reaction to a modification of the Sakaguchi test, applied directly to the chromatogram[6]. The amount of dinitrophenyl arginine present was determined after chromatography on talc, using a further modification of the Sakaguchi reaction[2]. Experiments with authentic dinitrophenyl arginine indicated that 75 per cent of the pure substance added to dinitrophenylated α-casein before the hydrolytic treatment, was recoverable at the end of the whole procedure. After applying a correction for this, the N-terminal arginine present in 10^5 gm. α-casein was calculated to be 3·8 moles. Previous values reported are 10·7[1], 1·8[2], and 3·6[3] moles per 10^5 gm. α-casein.

From these results it is concluded that the amount of lysine found as a terminal group is so small as not to be significant, and that α-casein has one type only of N-terminal amino-acid, arginine, in amount consistent with a minimum molecular weight for α-casein of approximately 26000.

It is hoped to publish a full account of this work elsewhere.

W. Manson

Biochemistry Department,
The Hannah Dairy Research Institute,
Ayr.

[1] Mellon, E. F., Korn, A. H., and Hoover, S. R., *J.A.C.S.*, **75**, 1675 (1953).
[2] Wissmann, H., and Nitschmann, H., *Helv. Chim. Acta*, **40**, 356 (1957).
[3] Ise, C. M., *Diss. Abs.*, **19**, 431 (1958).
[4] Waugh, D. F., and von Hippel, P. H., *J.A.C.S.*, **78**, 4576 (1956).
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[7] Blackburn, S., and Lowther, A. G., *Biochem. J.*, **48**, 126 (1951).

A Rapid and Sensitive Method for the Determination of Xanthine Oxidase Activity using the Oxygen Electrode

Fridovich and Handler have shown that xanthine oxidase at low concentration rapidly initiates the aerobic oxidation of sodium sulphite in the presence of hypoxanthine[1]. Xanthine oxidase is, notwithstanding, devoid of sulphite oxidase activity; but in the presence of a purine substrate becomes effective as a sulphite oxidase. These authors established that at constant concentration the rate of oxygen consumption is proportional to the xanthine oxidase concentration and the rate of oxygen uptake is proportional to the enzyme activity when at a constant level. Therefore, the measure of the sulphite oxidation is a very sensitive assay for xanthine oxidase.

The oxygen electrode has been employed extensively by Britton Chance for the study of oxidative phosphorylation and several reactions involving oxido-reduction mechanisms[2]. With this apparatus the oxygen uptake is easily followed and recorded immediately after the addition of the substrate to the reaction vessel, much more rapidly than can be shown by the manometric method[3].

It seemed to us that a very sensitive method for the determination of xanthine oxidase in tissue homogenates and of milk could be worked out using the sulphite oxidation method of Fridovich and Handler and measuring oxygen uptake with the oxygen electrode. It is the aim of this communication to propose an assay of this enzyme based on these methods. The oxygen electrode used in our experiments was constructed in Prof. Britton Chance's laboratory. The oxygen uptake was recorded in a chart calibrated in μM of oxygen per sec. The electrode is polarized at —0·6 V.[3,4]. Rat liver homogenates (1:3) were prepared either in 0·33 M sucrose or in 0·1 M phosphate buffer pH 7·8 in a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 min. at 800 g in a Sorvall centrifuge and the supernatant recentrifuged 20 min. at 10,000 g. The sediment was discarded since Villela *et al.* demonstrated that the xanthine oxidase activity is only present in the supernatant containing the soluble proteins, the remaining fractions being inactives[5].

Only rats weighing 70–100 gm., due to their low liver endogenous respiration, were selected. Preliminary assays showed that the endogenous respiration was practically eliminated if the homogenate was dialyzed for 24 hr. against a buffer phosphate pH 7·8 at 4°C. in 'Cellophane' bags. The experiments were performed in a volume of 2 ml. in the vessel reaction. Dialyzed liver homogenates (0·4 ml. containing 0·2–0·8 mgm. protein nitrogen) diluted with 1·4 ml. of phosphate buffer pH 7·8 were shaken with air to provide sufficient dissolved oxygen. After the stabilization of the electrode which is shown by a steady line in the graph 0·01 ml. of 0·1 M sulphite and 0·1 ml. of 5×10^{-5} M hypoxanthine were added to the vessel. The oxygen consumption was recorded in a drum (Esterline-Angus recorder) and calculated from the graph in μ moles per sec.

The effect of sulphite is slight when the endogenous purine substrate is low. Hypoxanthine added to the homogenate was slowly oxidized (in about 60 sec.) but increases rapidly after the addition of the sulphite. Sulphite alone is not oxidized unless the endogenous substrate for xanthine oxidase is high. Hypoxanthine and sulphite when added together

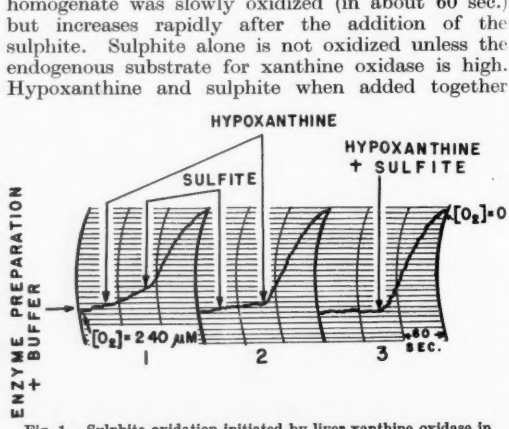

Fig. 1. Sulphite oxidation initiated by liver xanthine oxidase in the presence of hypoxanthine. 1, 0·1 M sulphite added after the equilibration of the electrode followed by the addition of 5×10^{-5} M hypoxanthine; total oxygen uptake = 646 μM/sec.; 2, hypoxanthine added after electrode equilibrated for 60 sec. and sulphite added 120 sec. later; oxygen uptake = 512 μM/sec.; 3, sulphite and hypoxanthine added at the same time; oxygen uptake = 738 μM/sec. Liver homogenate containing 0·32 mgm. protein nitrogen in phosphate buffer, sulphite 0·1 M and hypoxanthine $5 \times 10^{-5} M$, pH 7·8

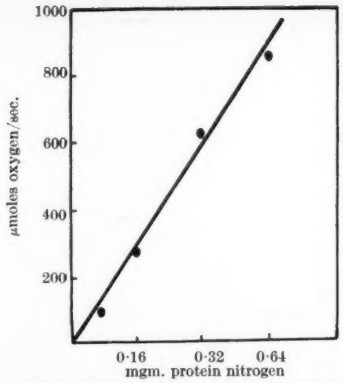

Fig. 2. Xanthine oxidase activity of dialyzed liver homogenate preparation. Oxygen consumption measured with the rotating oxygen electrode expressed in μM oxygen per sec. Hypoxanthine $5\times10^{-5}M$ and sodium sulphite 0·1 M added together to the reaction vessel containing the dialyzate diluted with phosphate buffer pH 7·8. Total volume 2 ml.

promote a rapid increase of the oxygen uptake. Fig. 1 summarizes these effects.

The sulphite oxidation initiated by xanthine oxidase in the presence of the purine substrate is proportional to the enzyme concentration in the liver homogenate. When graded volumes of the dialyzed homogenate were used, a straight line was obtained as shown in Fig. 2. This linear relationship holds only up to 0·6 ml. of the liver preparation equivalent to 0·64 mgm. of protein nitrogen. Active enzyme preparations should therefore be diluted or smaller quantities used.

We wish to thank Prof. Britton Chance, Johnson Foundation, University of Pennsylvania, Philadelphia, for the construction and calibration in his laboratory of the oxygen electrode used in the present work. One of us (M.B.) is grateful to the Rockefeller Foundation and Conselho Nacional de Pesquisas for generous grants.

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GILBERTO G. VILLELA
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Instituto Oswaldo Cruz,
Rio de Janeiro.
July 15.

[1] Fridovich, I., and Handler, P., *J. Biol. Chem.*, **233**, 1578 (1958).
[2] Chance, B., Harvey Lectures, Ser. 49, 145 (1953–1954).
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[4] Davies, P. W., and Brink, F. jun., *Rev. Sci., Instr.*, **13**, 524 (1942).
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Discrimination of Xylose from Arabinose and Some Other Sugars

IT has been found that the barbituric and thiobarbituric acids are suitable to discrimination of xylose from arabinose and other sugars. Good results have also been noted by using the spot test, as by spraying the developed chromatograms with the reagent. 0·5 gm. barbituric or 0·25 gm. thiobarbituric acid dissolved in 50 c.c. glacial acetic acid.

The dry paper bearing the sugar to be examined is sprayed with the reagent and placed for 15 min. at about 125°C. and then kept in the stream of steam.

The spot of xylose becomes green, arabinose and ribose brown, and the spots of other sugars (aldohexoses, ketohoxoses, 6-deoxyhexoses and the uronic acids) react with a variety of colours. The spots also show a different colour under ultra-violet illumination.

Full details of this work will be published in *Acta Biochimica Polonica*.

B. DROZDZ
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Pharmaceutical Faculty,
Medical Academy,
Poznan, Poland.

Periodate Technique for the Identification of Sugar Phosphates

THE mechanism of action of periodate on sugar phosphates has not been fully established and there is some controversy regarding the origin of the products of periodate action[1,2,3]. The convenient identification by chemical techniques of sugars esterified to phosphates at their terminal carbon atom depends on their oxidation by periodate to yield glycolaldehyde phosphate which may be identified by colorimetric procedures[4]. The extended periods required for theoretical periodate consumption coupled with the marked interference of both periodate and iodate in the conventional colorimetric method for glycolaldehyde determination (Fig. 1) have rendered this technique either equivocal or impractical. Treatment of aldose or ketose phosphates with sodium borohydride yields the corresponding polyol phosphates which are readily cleaved by periodate with theoretical consumption of oxidant generally occurring within 30 minutes.

One to ten micromoles of sugar phosphate are adjusted to pH 7·5 with 0·1 M *tris* (hydroxymethyl aminomethane) buffer and treated with 2 molar equivalents of sodium borohydride prepared in the same buffer. The mixture is allowed to stand for 30 min. at room temperature after which the pH is adjusted to 4·5 with 6 N acetic acid. The resulting solution is heated for three minutes in a boiling water bath to destroy excess borohydride and, after cooling, a 20 per cent molar excess of sodium periodate is added. Periodate consumption is most readily followed by a spectrophotometric procedure[5] and is generally complete in 15–30 min.

The solution is transferred to a one-ml. column of 'Dowex 1×8'-formate resin, 200–400 mesh, and the column washed with several volumes of water, and five volumes of 1 M formic acid. The glycoladehyde phosphate is eluted with 10 ml. of 0·5 N hydrochloric acid, the collection being made in a volumetric flask. An aliquot, corresponding to 0·1 μmole of sugar taken initially, is withdrawn for colorimetric estimation of glycolaldehyde content. The identity of the product may be confirmed by removal of the hydrochloric acid with silver carbonate, passage

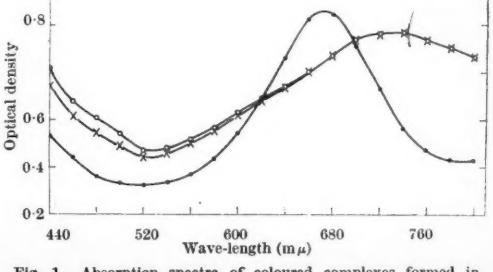

Fig. 1. Absorption spectra of coloured complexes formed in diphenylamine reaction (ref. 4) with iodate (○), periodate (×) and glycolaldehyde phosphate (●). Concentration is $2\times10^{4}M$ in each case

Table 1. Periodate Oxidation of Sugar Phosphates

Sugar	Glycolaldehyde Phosphate (% of theoretical)	Periodate Consumed (% of theoretical after 30 min.)
Glucose-6-phosphate*	105	104
Fructose-6-phosphate*	101	96
Ribose-5-phosphate*	91	90
Fructose diphosphate†	89	91
α-glycerophosphate*	100	98
Galactose-6-phosphate**	102	95

* Commercial product ; concentration based on organic phosphate content.

† Commercial product, yields two moles of glycolaldehyde phosphate per mole of sugar ; concentration based on organic phosphate content.

** Prepared according to unpublished procedure (Jacobson, B., and Davidson, E. A., in preparation) ; concentration based on organic phosphate content.

through a cation exchange resin to remove excess ions, concentration by lyophilization and chromatography according to the procedure of Pizer and Ballou[6]. The results with several model compounds are summarized in Table 1.

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Need for Inorganic Phosphate in Oxidations stimulated by Dinitrophenol

In an experiment with a phosphorylating kidney-cyclophorase preparation Loomis and Lipmann[1] discovered that 2,4-dinitrophenol could replace the inorganic phosphate necessary for a maximal rate of oxygen uptake with glutamate as the substrate. This observation was confirmed by Teply[2] and Judah and Williams-Ashman[3], using the same type of enzyme preparation, but the experiment has never been repeated, to our knowledge, with the purer and more tightly coupled mitochondrial preparations that have become available in the last few years. Since the observation of Loomis and Lipmann is of fundamental importance for present-day concepts of respiratory-chain phosphorylation (cf.ref. 4), we have re-investigated the problem with fresh rat-liver mitochondria using a medium with adenosine diphosphate, glucose and hexokinase to exhaust the endogenous inorganic phosphate of the mitochondria (cf. ref. 5). The result of one of our experiments is given in Fig. 1 and the mean figures obtained in eight experiments have been summarized in Table 1. It can be seen that under our conditions dinitrophenol gives only a small stimulation of the basal oxygen uptake and that phosphate is necessary to obtain maximal oxidation rates.

This inability of dinitrophenol to replace phosphate in glutamate oxidation was found with concentrations of 2,4-dinitrophenol varying between 5×10^{-6} and 10^{-3} *M* ; with *p*-nitrophenol, *p*-isoamyl-2, 6-dinitrophenol and gramicidin ; with rat-heart sarcosomes as well as with rat-liver mitochondria ; at *p*H values between 6·4 and 7·9 ; and at 37° as well as at 25°. It appears, therefore, that the original conclusion of Loomis and Lipmann—that dinitrophenol replaces inorganic phosphate in coupled glutamate oxidation does not hold for fresh rat-liver mitochondria and rat-heart sarcosomes and that it cannot be used as an argument in the discussion about possible mechanisms for respiratory-chain phosphorylation (cf. ref. 4).

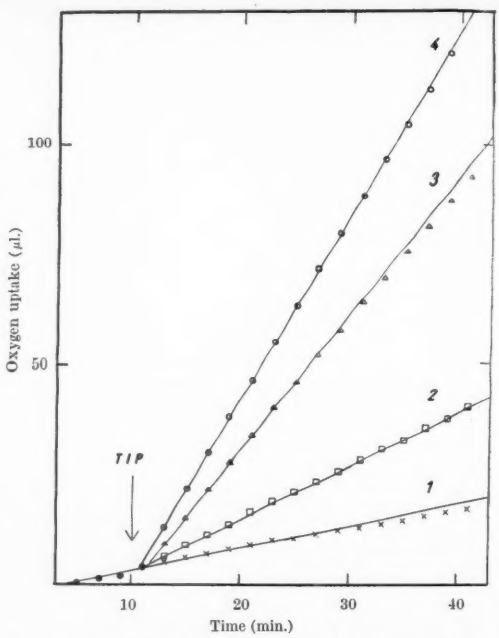

Fig. 1. The effect of 2,4-dinitrophenol and dinitrophenol + inorganic phosphate on the oxygen uptake of rat-liver mitochondria. The oxygen uptake was measured with differential manometers, the vessels containing 0·015 *M* potassium chloride, 0·002 *M* ethylenediaminetetraacetate, 0·05 *M tris* (hydroxymethyl) aminomethane-acetate buffer, *p*H 7·0, 0·005 *M* magnesium chloride, 0·0009 *M* adenosine diphosphate, 0·02 *M* glucose, 100–150 Cori units yeast hexokinase, 0·025 *M* sucrose, 0·01 *M* L-glutamatea nd 0·1 ml. fresh rat-liver mitochondria, containing 3·20 mgm. protein. Final volume, 1·0 ml. The reaction was started by the addition of the mitochondria ; after 10 min. the following additions were tipped in from the side-arm : 1, water ; 2, 0·1 μmole dinitrophenol ; 3, 20 μmoles inorganic phosphate ; 4, 0·1 μmole dinitrophenol + 20 μmoles inorganic phosphate. The temperature was 25°

The amount of inorganic phosphate necessary to raise the stimulation by dinitrophenol to half the maximal value is much less than that found by Chance[6] to be necessary for the oxidation of intramitochondrial reduced diphosphopyridine nucleotide. In Fig. 2, the percentage of the maximal stimulation is plotted against the phosphate concentrations tipped in together with dinitrophenol. It can be seen that the apparent Michaelis constant is less than 10^{-4} *M* inorganic phosphate. (It should be noted that the

Table 1. Stimulation of Respiration by Dinitrophenol in Presence and in Absence of Phosphate

	Mean	Standard Deviation	Range
Stimulation of the basal oxygen uptake by dinitrophenol alone	2·9	0·31	2·5–3·5
Stimulation of the basal oxygen uptake by dinitrophenol + inorganic phosphate	10·2	0·28	9·2–11·7
Oxygen uptake per mgm. protein per hr. in the presence of dinitrophenol alone	22·1	3·5	15·7–26·2
Oxygen uptake per mgm. protein per hr. in the presence of dinitrophenol + inorganic phosphate	77·4	5·9	69·0–84·7
Oxygen uptake in the presence of dinitrophenol alone as percentage of that found with dinitrophenol + inorganic phosphate	28·3	4·1	21·4–33·7

The values are calculated from eight consecutive experiments with different mitochondrial preparations. The conditions were the same as in Fig. 1 with two exceptions : in five experiments the glutamate concentration was 0·02 *M* instead of 0·01 *M*, and in four experiments the dinitrophenol concentration was higher than 10^{-4} *M*.

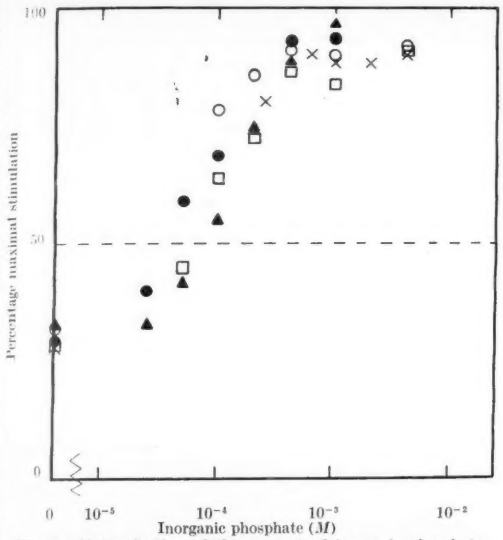

Fig. 2. Determination of the amount of inorganic phosphate necessary to obtain half-maximal stimulation by 2,4-dinitrophenol. (Maximal stimulation is defined as that given by 0·02 *M* phosphate.) The figure represents all the values found in five consecutive experiments with four different mitochondrial preparations. The conditions were the same as in Fig. 1, with one exception: in three experiments the glutamate concentration was 0·02 *M* instead of 0·01 *M*. ·, $7{\cdot}5\times10^{-5}$ *M* dinitrophenol; ×, $1{\cdot}25\times10^{-4}$ *M* dinitrophenol; □, $2{\cdot}5\times10^{-4}$ *M* dinitrophenol; △, 5×10^{-4} *M* dinitrophenol; ●, 5×10^{-4} *M* dinitrophenol. (The maximal rate was about equal for the two experiments (● and ○) performed with the same enzyme preparation, being 213·6 and 210·6 μl. per hr. respectively.)

values given for the percentage stimulation at phosphate concentrations below 10^{-4} *M* are probably too low, because at these concentrations the rates of oxygen uptake decline rapidly after adding the dinitrophenol + phosphate). The Michaelis constant determined in this way is much lower than would be expected from the inorganic phosphate concentrations that are actually found after stopping the reaction with trichloroacetic acid in the vessels with dinitrophenol alone. With Sumner's modification[7] of the Fiske-Subbarow method we obtained in four experiments a mean value of $1{\cdot}2\times10^{-4}$ *M* with a standard deviation of $0{\cdot}1\times10^{-4}$ *M*. Evidently the Fiske-Subbarow method determines a compound (or compounds) that is (are) not available as inorganic phosphate to the phosphate-requiring step in glutamate oxidation. The reason for this is not yet clear.

In order to locate the phosphate-dependent step in coupled glutamate oxidation, we have tested other substrates. Since a respiratory rate of $22{\cdot}1\pm3{\cdot}5$ μl. oxygen per mgm. protein per hr. can be obtained with glutamate and dinitrophenol in the absence of inorganic phosphate, only substrates that yield a rate in the presence of phosphate that is appreciably higher than this value could be expected to give useful results. Not all the intermediates of the Krebs cycle fulfil this criteria. A further complication was that concentrations of dinitrophenol, necessary to obtain complete uncoupling, inhibit the oxidation of several substrates. (This phenomenon is extensively discussed by Hülsmann[8].) However, with succinate oxidation conclusive results could be obtained: dinitrophenol stimulated the basal oxygen uptake about 6-fold, giving more than 90 per cent of the oxygen uptake in the presence of dinitrophenol and inorganic phosphate. Since the rate of oxygen uptake with succinate is at least twice as high as that with glutamate, it can be concluded that the phosphate-requiring step of coupled glutamate oxidation is in that portion of the respiratory chain lying between glutamate and cytochrome *b* and not in the subsequent oxidation of cytochrome *b*. Further details of these and related experiments will be submitted for publication at a later date.

We wish to express our thanks to Miss Betty Kelder for expert technical assistance and to Mr. A. G. Hilvers for collaborating in some of the experiments.

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Detection of 2:4-Dinitrophenyl Amino-acids on Paper Chromatograms

Aromatic nitro compounds have previously been detected on paper chromatograms by reduction, diazotisation and coupling with a phenol[1] as this method has not been found satisfactory for 2:4-dinitrophenyl derivatives, for example, 2:4-dinitrophenyl-amino-acids, two other methods which can be used in the presence of free amino-acids have been devised in addition to that of Grassmann and Riedel[2].

The first method which is of general application to aromatic nitro groups involves the reduction of the nitro group with titanous chloride and conversion of the amine to a coloured Schiff's base[3] by spraying with an acid solution of *p*-dimethylaminobenzaldehyde (Ehrlich's reagent). The chromatogram is sprayed with a solution of titanous chloride (15 per cent w/v (B.D.H. Ltd.) diluted 10 times with 0·5 *N*-hydrochloric acid) and heated for 15 min. at 60°. On spraying with a solution of Ehrlich's reagent (1 part of a solution of *p*-dimethylaminobenzaldehyde (0·33 per cent in hydrochloric acid (50 per cent v/v) (in 2 parts ethanol) nitro compounds appear immediately as yellow-orange spots which soon fade and reappear in 10–15 hr.

p-Dimethylaminobenzaldehyde is known to react with many compounds other than aromatic amines. It reacts with pyrrole and its derivatives (the reaction with amino-sugars and N-acetylated amino-sugars probably depends on the formation of a pyrrole chromogen from the sugar which then reacts with the Ehrlich's reagent[4]), urea, citrulline[5], and hydrazine[6]. The reactivity of aliphatic amines and amino-acids is dubious. Frehden and Goldschmidt[3] report that both these classes of compounds react in glacial acetic acid to form coloured derivatives, but Pesez and Petit[6] could detect no reaction in dilute hydrochloric acid. The α-amino-acids (other than those with reactive side chains, for example, tryptophane) do not form brightly coloured derivatives with the reagent on chromatograms. In accordance with the report of Dalgliesh[7] it has been found that these amino-acids develop a very pale yellow colour after several days.

Nitro compounds can be distinguished from these other compounds as they only react after reduction.

(Clearly, nitro groups cannot be distinguished from other oxidation products of amines by this method.) The method has been successfully used for the detection on paper chromatograms of colourless 2:4-dinitrophenyl-amino-acids, for example, O-2:4-dinitrophenyl-tyrosine and *im*-2:4-dinitrophenyl-histidine in acid hydrolysates of 2:4-dinitrophenyl-proteins; as little as 2–3 μgm. can be detected. The technique is now being developed for the quantitative estimation of these compounds.

The second method is based on a reaction between potassium cyanide and 2:4-dinitrophenyl derivatives which is claimed by Anger[8] to be specific for the latter. The chromatogram is dipped in an aqueous solution of potassium cyanide (8 per cent w/v) and heated for 2 minutes at 90°. 2:4-dinitrophenyl derivatives produce red-violet spots. 2–3 μgm. can be detected. The method is of value in the confirmation of the identity of the compounds but the toxicity of the cyanide is a disadvantage for routine use.

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Antagonism of the Oxidative Dissimilation of *p*-Aminobenzoic Acid by *p*-Aminosalicylic Acid

p-Aminobenzoic acid is essential as a growth factor in various organisms, and this observation has stimulated extensive investigations involving antagonists of this metabolite and possible modes of action. Antimetabolites such as sulphanilamide[1,2] *p*-nitrobenzoic acid[3,4] and *p*-aminosalicylic acid[5] have demonstrated antagonistic activities. In addition to functioning as a growth factor, *p*-aminobenzoic acid may also serve as an oxidizable substrate for *Pseudomonas fluorescens*[6]. Several structurally related compounds were tested to determine if they influenced the oxidation of this compound. Of the three compounds previously reported to be antagonistic, only *p*-aminosalicylic acid influenced the oxidative dissimilation of *p*-aminobenzoic acid[7]. The inhibition by *p*-aminosalicylic acid appeared to be competitive since *p*-aminobenzoic acid was oxidized to the same extent in the presence and absence of *p*-aminosalicylic acid and the inhibitor could be displaced by increasing the substrate concentration[7].

Studies have revealed that the oxidation of *p*-aminobenzoic acid by an organism tentatively identified as a *Flavobacterium* is also competitively inhibited by *p*-aminosalicylic acid. This investigation was undertaken to elucidate the mechanism of this antagonism.

Investigations were conducted to study the disappearance of the substrate from the medium in the presence and absence of *p*-aminosalicylic acid. Cells for experimental studies were grown on a synthetic salts medium containing *p*-aminobenzoic acid as the sole source of carbon and energy and harvested as previously described[7]. The cells were incubated with constant shaking at 37°C. in the presence and absence of varying ratios of the inhibitor. Aliquots were taken, the cells removed by filtration through a millipore filter, and the filtrate assayed for the substrate.

p-Aminobenzoic acid was determined by modification of a previously described procedure[8]. The coupling reaction was performed by taking a 0·5 ml. aliquot of the filtrate, diluting to a total volume of 3·5 ml., and adding the following reagents: 1·0 ml. of a 7·5 per cent trichloroacetic acid, shake and allow to stand for 5 min.; 1·0 ml. of 0·025 per cent sodium nitrite and stand 20 min.; 1·0 ml. of 0·5 per cent ammonium sulphamate, stand for 20 min.; and 2·0 ml. of 0·0625 per cent N-(1-naphthyl)-ethylenediamine dihydrochloride. The colour was permitted to develop for 40 min. after which the sample was diluted with 15 ml. of distilled water and the percentage transmittance read in a spectrophotometer at 560 mμ. Values were then calculated from a standard curve prepared concurrently. Controls indicated that *p*-aminosalicylic acid, in the concentrations employed in this investigation, did not influence this test.

The uptake of *p*-aminobenzoic acid by the cell suspension was followed in different substrate inhibitor ratios. The results presented in Table 1 are representative of the information obtained in this study. These results show that the substrate (*p*-aminobenzoic acid) is depleted from the medium in 45 min. in the absence of the inhibitor. In the presence of *p*-aminosalicylic acid the substrate is taken up by the cells at a much slower rate. The data indicated that the rate of uptake is a function of the substrate inhibitor ratio, since depletion of the substrate from the medium is much slower in the higher ratios than in the lower ratios. It was interesting to note that in the presence of the inhibitor the initial uptake during the first time period was greater than the uptake during subsequent time intervals, and after this initial uptake there was little, if any, additional substrate depleted from the medium in the 1/10 ratio.

Table 1. Cellular uptake of *p*-aminobenzoic acid from the medium in the presence and absence of varying ratios of *p*-aminosalicylic acid

Time (min.)	Concentration (μgm.) of *p*-aminobenzoic acid remaining in filtrate			
	Ratio: *p*-aminobenzoic acid/*p*-aminosalicylic acid			
	1/0	1/3	1/5	1/10
0	58·4	58·0	57·3	58·0
15	40·0	43·6	48·9	52·6
30	17·3	37·0	45·9	49·5
45	0·0	23·3	38·3	50·0

It is suggested that *p*-aminosalicylic acid may influence the oxidative dissimilation of *p*-aminobenzoic acid by competing with the substrate for the transport mechanism(s), thereby controlling oxidation by regulating intracellular accumulation of substrate.

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Chromium Ribonucleoprotein from Bovine Liver

We have recently isolated from beef liver a ribonucleoprotein containing 0·1 per cent of chromium.

Chromium ($Z=24$), manganese ($Z=25$) and nickel ($Z=28$) are the only metals of atomic numbers between 23 (vanadium) and 30 (zinc) which have not been shown to exhibit biological functions in association with specific organic substances *in vivo*. The atomic structure and chemical reactivities of these three elements, however, have many similarities to those of vanadium ($Z=23$), iron ($Z=26$), cobalt ($Z=27$), copper ($Z=29$), and zinc ($Z=30$), the elements of the first transition group of the periodic table known to have distinct biological activities in conjunction with specific biological products. The abundance of manganese and chromium in the biosphere actually exceeds that of all these, except iron. Such circumstances have led to the conjecture that these elements would yet be detected in discrete biological compounds with specific biological roles[1].

On this basis we sought to identify an organometallic substance containing chromium. A first suggestion that such a complex exists arose from the discovery that one of the intermediate fractions in the isolation of beef liver glutamic dehydrogenase, the precipitate obtained after the addition of 28 per cent sodium sulphate, reproducibly contained substantial aggregations of chromium varying from 22 to 40 μgm./gm. dry weight, nearly one thousand fold enrichment of this metal over its concentration in beef liver, 0·05 μgm./gm. dry weight[2,3].

In the further isolation of this chromium-containing substance the procedure employed for the isolation of beef liver glutamic dehydrogenase was followed[4]. However, after the second precipitation at *p*H 5·8, the resulting precipitate was dissolved in 0·05 *M* potassium phosphate, *p*H 7·4 and instead of sodium sulphate, ammonium sulphate was added in stepwise fashion, in increments of 10 up to 90 per cent saturation. After each addition, the precipitated material was removed by centrifugation, and both the precipitates and the supernatant solutions were analysed for chromium. In each instance the major portion of the chromium remained in the supernatant fraction. The supernatant fraction, after the addition of ammonium sulphate to bring it to 90 per cent saturation, was dialysed against 0·05 *M* potassium phosphate buffer and lyophilized. The chromium contents of the various fractions are shown in Table 1. The final material contained 1080 μgm. of chromium per gm. dry weight, a 20,000-fold enrichment over the concentration of this metal in whole liver. The pronounced maximal absorption at 258 mμ of the lyophilized material, dissolved in 0·05 *M* potassium phosphate, *p*H 7·4, suggested the presence of a nucleic acid. The material was shown to be composed of 70 per cent ribonucleic acid, measured by the orcinol method[6], 30 per cent protein[6] and 4·0 per cent phosphorus[7]. The substance did not react with diphenylamine, indicating that deoxyribonucleic acid was absent[8]. The lyophilized material was dissolved and dialyzed for 48 hr. against metal-free water. Chromium was not removed. While chromium is here singled out for discussion because of the unique nature of its occurrence, spectrographic analysis of this dialysed material demonstrated that in addition to chromium it also contained very substantial quantities of calcium, magnesium, strontium, barium, aluminium, iron, nickel and zinc (Table 2).

Table 1. Chromium Content of Fractions obtained in the Isolation of a Ribonucleoprotein from Beef Liver

Mainline Fractions	Chromium in μgm./gm. dry weight	Discard Fractions	Chromium in μgm./gm. dry weight
Whole liver	0·05	Precipitate *A*	3·7
Homogenate	1·7	Supernatant *B*	3·1
Supernatant *A* (20% ethanol)	5·8	Supernatant *C*	8·0
Precipitate *B* (*p*H 5·8)	7·9	50% Ammonium sulphate precipitate	23·0
Precipitate *C* (5% ethanol, *p*H 5·8)	12·0	90% Ammonium sulphate precipitate	15·5
50% Ammonium sulphate supernatant	240·0		
90% Ammonium sulphate supernatant	1080·0		

Table 2. Emission Spectrographic Analysis of Ribonucleoprotein from Beef Liver

Metal	Content in μgm./gm. dry weight	Metal	Content in μgm./gm. dry weight
Magnesium	7880	Aluminium	930
Calcium	13000	Iron	1540
Strontium	97	Chromium	1080
Barium	350	Nickel	2340
		Zinc	4950

To our knowledge the demonstration of the association of chromium and nickel with this ribonucleoprotein represents the first instance in which these metals have been found to be associated firmly with a specific native biological product. These two metals, like the other members of the first transition group of the periodic table, may therefore perform unique biological functions in conjunction with specific biological compounds.

The occurrence of these and other metals in this location suggests that they play a part in the function of nucleic acids, either in the maintenance or their structure or to provide specific sites of binding between proteins and nucleic acids. Metals are known to serve in the maintenance of protein structure, apparently through their ability to form strong covalent bonds with organic ligands[9–11]; chromium, in fact, has been reported to be exceptional in its capacity to act as a 'cross-linking' intermediate between individual protein molecules, such as collagen and conarachin[12,13]. We have considered the possibility that some of these metals may stabilize acid structure through the formation of 'sandwich complexes'[14], while some may serve to link nucleic acids to protein.

The discovery of metals in this ribonucleoprotein has led to the examination of the metal content of nucleic acids from a wide variety of phyla and species, all of these contain large amounts of the same metals. The details of this work and its implications for the biological function of nucleic acids will be reported elsewhere.

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Some Enzymatic Properties of Suspensions of Parenchymatous Liver Cells

The heterogeneous population of rat liver[1] has led to a discussion of the interpretation of enzyme activities based on whole liver or whole liver proteins[2]. Recent interest in the properties of suspensions of parenchymatous liver cells[3,4] has raised the possibility of using such cells as reference base for enzyme determinations. By shaking rat liver slices in calcium-free citrated Krebs-Ringer phosphate buffer with added glucose, passing the slices through graded sieves to break them into the component cells, centrifuging and resuspending the sediment, we have shown[5,6] that the cells of such suspensions leak most of their content of glutamic pyruvic transaminase while retaining glutamic oxalacetic transaminase. Subsequent studies have shown that in general between 70 and 90 per cent of lactic acid dehydrogenase, 'TPN-linked' isocitric dehydrogenase and glucose-6-phosphate dehydrogenase are similarly lost from the cells in the course of making the preparation. The leakage of lactic acid dehydrogenase was not prevented by making the medium between 0·01 *M* and 1 *M* in pyruvate both with and without the addition of 2–5 per cent bovine albumen fraction V, by the presence of adenosine triphosphate (750 mgm. per cent) crystalline human oxyhæmoglobin (5–10 per cent) and carbonic anhydrase, the latter two being used both singly and in combination. By making the medium 20 per cent in washed human red blood cells the leakage appeared to be inhibited[7].

Studies with Evans blue have shown, however, that this could be accounted for by the amount of supernatant trapped in the loosely packed sediment. The leakage of enzyme could not be inhibited either by increasing the concentration of glucose to 500 mgm. per cent and using a medium containing amino-acids, with the addition of insulin, α-keloglutarate, adenosine and α-tocopherol which have been found to maintain oxydative phosphorylation in perfused rat liver[8], or by substituting ethylene diamine tetra-acetate for citrate in equimolar amounts. Likewise, the addition to the medium of whole liver homogenate prepared from a remainder of the rat liver used in the preparation of the cells did not result in retention of enzyme by the cells if the cells were washed once by resuspending in fresh medium and centrifugation. This was found to be the case whether pyruvate was added to the medium or not. Studies of the leakage from rat liver slices shaken for a period of 1 hr. at 37°C. showed that on an average only 50 per cent of the enzyme (expressed on a protein basis) remained in the slices at the end of that time. This was found at *p*H 5·0, 6·0, 7·0 and 7·3, and for a molarity varying from 0·3 *M* to 1·8 *M*.

Two of the particulate enzymes, glutamic dehydrogenase and glucose-6-phosphatase, remained in the cells in the proportion expected if no leakage had occurred (Table 1).

It, therefore, appears that of the predominately 'soluble' enzymes so far tested only glutamic oxalacetic transaminase remained within the cell. The overall recovery of protein from the cells and from the supernatants was more than 90 per cent and the amount of protein per cell was generally between 80 and 90 per cent. The majority of the protein component of the cell is, therefore, retained. The amount of cellular protein which has enzymatic activity is not known with certainty but has been estimated as being about 60 per cent by Lang[9]. Between one-half and one-third of the cellular protein is in the 'soluble fraction' of the cell[10]. If it is further assumed that almost all the non-enzymic cellular protein is in the particulate fraction, the average of 10–20 per cent of protein lost from the cell could be accounted for largely by enzymic protein from the 'soluble' fraction.

The activities of glutamic oxalacetic transaminase, glutamic dehydrogenase and glucose-6-phosphatase in rat liver can, however, be expressed on a cellular basis and are given in Table 2.

Further work on enzyme leakage from morphologically intact liver cells is in progress.

Our thanks are due to Dr. H. S. Wiggins for constant help and advice and to the Michigan State Board of Alcoholism and the Horace H. Rackham School of Graduate Studies for grants in support of this work.

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Table 1. Enzyme Activities in Liver Cells

	Glucose-6-phosphatase			Glutamic dehydrogenase (Medium containing 2% bovine albumen fraction V)		
	Enzyme/cell (%)*	Enzyme per cel protein** (%)	Total Recovery of enzyme† (%)	Enzyme per cell (%)*	Enzyme per cell protein** (%)	Total recovery of enzyme† (%)
Mean	115·9	100·9	67·2	95·7	122·2	93·9
S.D.	17·2	14·1	13·3	13·2	21·4	7·6
No. of obs.	8	9	9	8	8	8

*Activity in cell suspension / Activity in control × cell yield × 100 (corrected for weight) where cell yield = parenchymatous cells per mgm. wet wt. of liver used in preparation / 120,000 (ref. 1)

**Specific activity of cell suspension / Specific activity of control homogenate × 100

†Activity of cell suspension/activity of media / Activity of control × 100 (corrected for weight)

Note: Low total recovery of glucose-6-phosphatase was found to be due to loss of enzyme in the medium.

Table 2. Enzyme Activities on a Cellular Basis (μmol./10^7 cells/hr.) at 37C.

Enzyme	Activity	S.D.	No. of obs.
Glutamic oxalacetic transaminase	719	230	16
Glucose-6-phosphatase	0·063	0·013	6
Glutamic dehydrogenase	96·3	23	8

Attempts to Modify the Normal Metabolism of Strontium-90 in Rats

Ito *et al.*[1] and Lindenbaum[2] injected the sodium or potassium salt of rhodizonic acid in order to increase the excretion of strontium-90 in rats. The former observed a slowing, the latter an acceleration in the elimination of strontium-90. We found that sodium rhodizonate given parenterally slows the removal of strontium-90 from the body, mainly by depressing the urinary excretion of radiostrontium. The effect was dependent upon the amount of rhodizonate injected (Fig. 1).

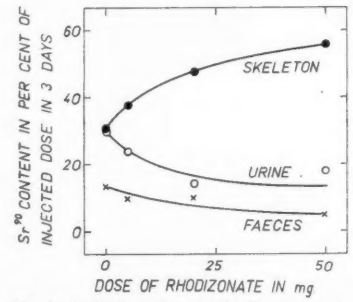

Fig. 1. Effect of rhodizonate on retention and excretion of strontium-90 in the rat. Each point represents mean from 5 rats in control group and from 2 rats each for three treated groups. Total skeletal content was calculated by multiplying the content of both femurs by a factor of ten. Each animal received about 2 μc. of strontium-90 with 0·5 mgm. of strontium chloride carrier.

In further preliminary experiments we tried to modify the fæcal excretion of strontium-90. In the first study we injected strontium-90 intraperitoneally and administered barium sulphate or tertiary calcium phosphate orally one hour before and immediately before strontium-90 injection. The amount of strontium-90 found in the fæces of the treated group after 72 hours was about 50 per cent above the control levels. The urinary excretion of strontium-90 remained unchanged after barium sulphate, but after the administration of calcium phosphate less strontium-90 was excreted in urine. The total radiostrontium excretion in both cases did not change essentially. Therefore, another experiment was performed where zirconium citrate, which is known to stimulate the urinary excretion of radiostrontium[3,4] was administered intraperitoneally half-an-hour before strontium-90, together with tertiary calcium phosphate, given orally one hour before and immediately before strontium-90. Radiostrontium was injected subcutaneously to prevent its forming a direct complex with zirconium citrate in the peritoneal cavity. The results are shown in Fig. 2. The effect of zirconium citrate in promoting excretion of strontium-90 in urine was predominant, but calcium phosphate did not stimulate fæcal excretion of strontium-90 when administered in combination with zirconium citrate. The effectiveness of calcium phosphate given alone in increasing the excretion of radiostrontium through the gut was confirmed in the present experiment.

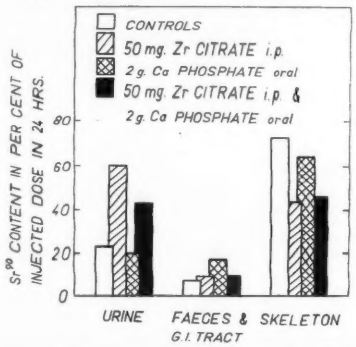

Fig. 2. Effect of tertiary calcium phosphate and zirconium citrate on retention and excretion of strontium-90 in the rat. Values are expressed as mean of 3 rats per group. Total skeletal content was calculated by multiplying the content of both femurs by a factor of ten. Each animal received intraperitoneally about 2 μc. of strontium-90 with 0·5 mgm. of strontium chloride carrier.

Finally, we studied the possibility of decreasing the intestinal absorption of strontium-90 using tertiary calcium phosphate. Strontium-90 was given to fasting animals by stomach tube, followed immediately by the calcium phosphate suspension, administered in the same way. Only 13 per cent and 25 per cent of the strontium-90 administered was found in the femurs of the treated animals after 4 hours and 8 days respectively, as comparing with the controls. This is even less than in the similar experiments of MacDonald *et al.*[5], who used tertiary sodium phosphate.

Thus, in the experiments described using barium sulphate and tertiary calcium phosphate, we were able to modify the transfer of strontium-90 through the intestinal wall; promoting the elimination of absorbed strontium-90 from the blood to the gut and its excretion with the stools, and diminishing the absorption of strontium-90 from the gut to the blood and its deposition in the bones. For this purpose it would also be possible to use other unabsorbable materials, for example, complexing agents, which cannot be given parenterally.

The details of this work will be published elsewhere.

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Role of Magnesium in the Insulin-Sensitivity of the Rat Diaphragm *in vitro*

It was reported earlier[1] that insulin increases the uptake of glucose by the isolated rat diaphragm only in the presence of moderate amounts of certain metallic ions, such as sodium, potassium, rubidium, cæsium and magnesium. The present communication reports the results of some further experiments which stress the importance of the magnesium ion with respect to the insulin-sensitivity of the rat diaphragm.

Diaphragms from overnight fasted rats (weight 100–120 gm.) were first incubated for ½ hour in media of different compositions without any added substrate. The effect of this pre-incubation on the insulin-sensitivity of the tissue, as measured by increased uptake of glucose in the presence of insulin from Geys' salt mixture[2], was then tested. Both the initial and final incubations were carried out under aerobic conditions.

The results given in Table 1 show that insulin caused the usual increase in the uptake of glucose by a diaphragm which had been pre-incubated for ½ hour in Geys' buffer either at room temperature (20° C.)

Table 1. Effect of ½ Hour's Pre-Incubation in Sucrose-Bicarbonate Buffer* on the Insulin-Sensitivity of the Rat Diaphragm

Pre-incubation†	Glucose uptake‡ (mgm./hr./gm. of wet tissue)	
	Without insulin	With insulin (0·1 unit/ml.)
None	3·39 ± 0·18§	8·41 ± 0·38
In Geys's buffer at room temp.	3·60 ± 0·15	8·18 ± 0·41
In Geys's buffer at 38° C.	3·81 ± 0·37	7·58 ± 0·39
In sucrose-bicarbonate buffer at room temp.	3·51 ± 0·35	9·9 ± 0·34
In sucrose-bicarbonate buffer at 38° C.	4·09 ± 0·13	5·73 ± 0·22

* This buffer consisted of a mixture of isotonic sucrose and isotonic sodium bicarbonate in which the final strength of the bicarbonate was 0·02 *M*.

† Pre-incubation at 38° C. was carried out by suspending 1 diaphragm in 2 ml. of the medium and shaking at 38° C. in a bath under 95 per cent oxygen + 5 per cent carbon dioxide. Pre-incubation at room temperature was carried out by suspending 6 diaphragms in 12 ml. of the medium in a cylinder. A stream of 95 per cent oxygen + 5 per cent carbon dioxide was bubbled through the medium so that the diaphragms were vigorously agitated.

‡ One hemidiaphragm was suspended in 1 ml. of Geys's medium, containing about 200 mgm. per cent of glucose, and shaken at 38° C. for 1 hour under 95 per cent oxygen + 5 per cent carbon dioxide. There were 6 hemidiaphragms in each group.

§ Standard error of the mean.

or at 38° C. The ability of the tissue to respond to insulin was also left unimpaired when it was pre-incubated for ½ hour at room temperature in sucrose-bicarbonate buffer. Pre-incubation for ½ hour at 38° C. in sucrose-bicarbonate buffer, however, caused a marked loss of the insulin-sensitivity of the diaphragm. It was also observed that when the excised diaphragm was put into the sucrose-bicarbonate buffer at room temperature, it began to contract vigorously after a few minutes. The diaphragm put into Geys's buffer did not show this phenomenon.

Table 2. Effect of the Addition of Low Concentrations of Magnesium, Calcium and Manganese to Sucrose-Bicarbonate Buffer* on the Loss of Insulin-Sensitivity of Rat Diaphragm due to Pre-incubation at 38° C.

Additions	Glucose uptake‡ (mgm./hr./gm. of wet tissue)	
	Without insulin	With insulin (0·1 unit/ml.)
None	3·81 ± 0·19‡	5·86 ± 0·35
4×10^{-3} *M* $MgCl_2$	3·89 ± 0·29	7·87 ± 0·29
1×10^{-3} *M* $MgCl_2$	4·39 ± 0·19	8·14 ± 0·29
2×10^{-4} *M* $MgCl_2$	4·64 ± 0·17	6·84 ± 0·23
1×10^{-3} *M* $MgCl_2$ + 1×10^{-3} *M* $CaCl_2$	2·71 ± 0·47	7·36 ± 0·39
1×10^{-3} *M* $MgCl_2$ + 5×10^{-3} *M* $CaCl_2$	3·81 ± 0·10	5·13 ± 0·34
5×10^{-3} *M* $MgCl_2$ + 5×10^{-3} *M* $CaCl_2$	3·73 ± 0·29	6·77 ± 0·32
4×10^{-3} *M* $MnCl_2$	3·57 ± 0·19	5·11 ± 0·14
1×10^{-3} *M* $MnCl_2$	4·49 ± 0·16	6·02 ± 0·24
1×10^{-4} *M* $MnCl_2$	3·67 ± 0·16	5·44 ± 0·35

* See under Table 1.
† See under Table 1.
‡ Standard error of the mean.

Table 2 gives the results of the addition of low concentrations of magnesium, calcium and manganese during pre-incubation of the diaphragm at 38° C. in sucrose-bicarbonate buffer. The results show that low concentrations of magnesium completely protected the tissue against loss of insulin-sensitivity due to the pre-incubation. They also show that this effect of magnesium was counteracted by suitable concentrations of calcium, and that the inhibitory effect of calcium could be overcome by increasing the concentration of magnesium in the medium. Manganese ($4 \times 10^{-3}M$ to $1 \times 10^{-4}M$) could not protect the tissue against loss of insulin-sensitivity due to the pre-incubation. It was also observed that addition of magnesium to the medium in amounts which protected the tissue against loss of insulin-sensitivity also stopped the contraction mentioned above.

From the foregoing results it appears that the insulin-sensitivity of the rat diaphragm is possibly directly controlled by the activity of some magnesium-adenosine triphosphate-linked enzyme in the system.

I wish to thank Prof. F. G. Young for his continued interest in this study. My thanks are also due to the managers of the I.C.I. Fellowships Fund, University of Cambridge for the grant of a Fellowship. The crystalline insulin used in the above experiments was a gift from the Wellcome Foundation, London, through the courtesy of Dr. C. G. Pope.

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GENERAL PHYSIOLOGY

Electron Spin Resonance Investigations of *Allium cepa* and *Calliphora vomitoria*

Recent publications of Commoner, Townsend and Pake[1] and Truby and Goldzieher[2] have shown the value of electron-spin resonance studies in detecting the presence of free radicals in biological tissues. During the course of a survey of free radicals in biological tissues we noted that two tissues in particular gave strong signals. These were seeds of *Allium cepa*, the onion, and larvæ and adults from *Calliphora vomitoria*, the blow fly.

Seeds of the onion, *Allium cepa* cooled to 90°K. gave a strong electron spin resonance signal to a carrier wave of 9,000 Mc./s. and a magnetic field of 3,000 gauss (Fig. 1*a*). The seeds were then hydrated and allowed to germinate, the signal strength being tested by serial samples taken at specific times after hydration. The signal for 15 comparable seeds progressively decreased reaching a minimum 10 hr. after hydration. This was followed by a maximum signal some 25 hr. after hydration. Finally there was another minimum 54 hr. after hydration (Fig. 1*a*–*e*; 1*f*). Though it is not yet possible to state the reasons for the observed minima and maxima, it is possible that they could be correlated with the change in oxygen concentration within the seed following germination; the maximum 25 hr. after germination following the breaking of the seed coat and the free entry of oxygen.

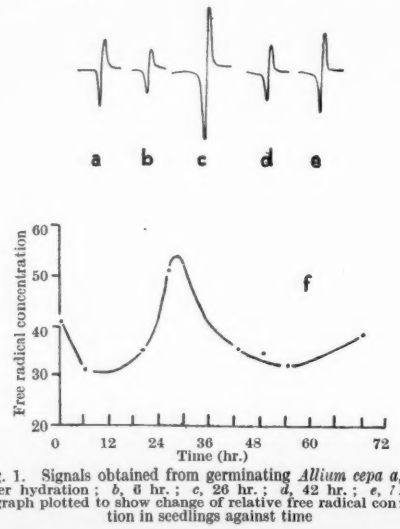

Fig. 1. Signals obtained from germinating *Allium cepa* *a*, 0 hr. after hydration; *b*, 6 hr.; *c*, 26 hr.; *d*, 42 hr.; *e*, 72 hr.; *f*, graph plotted to show change of relative free radical concentration in seedlings against time

Young larvæ from *Calliphora vomitoria* gave a weak signal, but the signal was larger when older larvæ were examined and larger still when pupæ and adults of equivalent weight were placed in the resonance cavity. We initially thought that this might have been due to free radicals present in rapidly growing tissues but the fact that the adults gave the largest signal indicated that another factor might be important. This was confirmed when we found that adult *Calliphora* still gave a strong signal after being dead for several hours at room temperature. The progression larvæ—pupæ—adult shows an increase in pigmentation, and it is possible that the melanins responsible for the pigmentation are responsible for the signals. This was partially confirmed by the fact that the melanins when extracted from adult flies gave a good signal as shown in Fig. 2e.

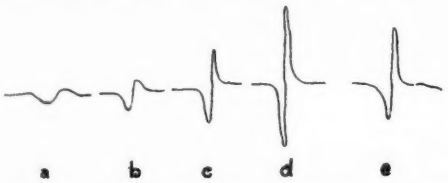

Fig. 2. Signals obtained from *Calliphora vomitoria*. *a*, from young maggots; *b*, from pupæ; *c*, from adult; *d*, from adults that had been dead at room temperature for 12 hr.; *e*, from melanin extracted from adult *Calliphora*

These two sets of results, though of a preliminary nature, indicate the value of electron spin resonance studies as a tool in biological research. Full details of this work will be published at a later date.

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ANIMAL PHYSIOLOGY

Effects of Elevated Plasma Sodium Concentration on Sodium and Potassium in the Erythrocyte of the Lizard *Trachysaurus rugosus* (Gray)

The lizard *Trachysaurus rugosus* has been shown to tolerate very high plasma sodium concentrations, for example, 240 m. equiv./l., both in its natural habitat during summer and in the laboratory after the injection of sodium chloride solution[1]. The injected sodium chlorate reached osmotic equilibrium with the total body water but electrolyte changes inside the cells were not determined. The nucleated erythrocytes of *Trachysaurus* offered a useful starting point for intracellular studies *in vivo*. It has been shown *in vitro* that the sodium concentration in the nucleated erythrocytes of the turtle is actively maintained by respiration[2].

Intraperitoneal injections (1 gm./100 gm./body-weight) or a 6 per cent (w/v) sodium chloride solution were given to the animals on each of six successive days. Blood samples, 2 ml., were collected by heart puncture into heparinized syringes. Sodium and potassium concentrations in the cells were calculated from their concentration in whole blood and in plasma and the hæmatocrit values[3,4]. Electrolyte concentrations were determined with an *EEL*-flame photometer. Cell water was calculated from water content of the whole blood and plasma and hæmatocrit value; dry weights being determined after 24 hr. at 105° C. The animals were kept at 21° C. and given nothing to eat or drink during the experiment.

After the second injection of sodium chloride solution no water had been lost from the erythrocytes, but both sodium and potassium concentrations inside the cells had risen, sodium more than potassium. The small decrease in hæmatocrit would be consistent with the removal of two ml. of blood in the initial sample. After the sixth injection of sodium chloride solution, the hæmatocrit had decreased much more than would be expected from the blood samples previously taken. The values for cell water in Table 1 are expressed as w/v. The change in w/v between the 2nd and 6th day corresponds to a 23 per cent decrease in cell volume. This would explain a substantial part of the change in hæmatocrit and would correspond to a loss of 30 per cent of the original content of water. Despite such a degree of cellular dehydration, the sodium concentration in the cell water was no higher than on the second day and sodium content of the cells must therefore have been diminished. This suggests an active extrusion of sodium. On the other hand the intracellular potassium concentration rose, and this increase could be satisfactorily explained by the observed loss, presumably osmotic, of cell water with little or no change in the cellular content of potassium. Detailed results are given in Table 1.

Table 1. Effects of Sodium Chloride Injections on Sodium and Potassium Concentrations in the Erythrocyte and Plasma

	Hæmatocrit	Cell water	Plasma m. equiv. l./water		Cell m. equiv./l. water		Difference between Plasma and Cell Na + K
		w/v	Na	K	Na	K	
Initial	26·2 ± 1·47	75·74 ± 1·11	167·7 ± 2·72	4·77 ± 0·17	49·8 ± 5·0	124·7 ± 2·92	2·1 ± 7·43
24 hr. after 2nd inject.	24·3 ± 1·48	75·69 ± 1·12	192·1 ± 3·15	4·25 ± 0·25	86·6 ± 9·37	142·8 ± 3·42	34·8 ± 10·92
24 hr. after 6th inject.	15·1 ± 1·04	68·56 ± 2·25	250·3 ± 2·24	3·60 ± 0·22	85·0 ± 6·41	196·4 ± 3·30	27·6 ± 10·06

Values are the means ± *S.E.* for the same ten animals, mean weight 456 gm. (range 380–618 gm.)

Initially the sum of the concentration of the cations sodium and potassium was the same in the cell water as in the plasma water. After both the second and sixth injection of sodium chloride solution the intracellular concentrations exceeded those in the plasma. Assuming osmotic equality of the two fluids this indicates an increased dissociation of polyvalent organic anions in the cells. Excess chloride in the plasma might be compensated by decrease in bicarbonate. In rabbits, it has been shown that plasma bicarbonate concentrations diminish after injection of hypertonic sodium chloride solution[5].

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Effect of 'Amino-acid Imbalance' on Growth and Vitamin A Storage in the White Rat

In the course of the studies concerning the interrelationship of leucine and norleucine[1,2,3] it was observed that rats fed low protein diets supplemented with norleucine or norleucine and leucine were retarded. When the animals were killed and their livers analyzed for vitamin A content, it was found that these animals invariably stored a lower amount of vitamin A when compared with the control group of animals (Table 1). In view of the fact that the inhibition of growth was always associated with a depressed appetite, it was of considerable interest to determine whether the low liver vitamin A values were a consequence of the reduced food intake and thus low vitamin A consumption or whether they resulted from apparent 'amino-acid imbalance'. In order to determine this a study was made in which the vitamin A intake was controlled.

Female weanling albino rats of the Sprague-Dawley strain were fed the appropriate diets, as indicated in Table 2, *ad libitum*, for a period of 3 weeks. At the end of this period the animals were killed and their livers analyzed for vitamin A content by the method of Ames *et al*[4]. The basal diet free of vitamin A contained 9 per cent casein (vitamin-free), 15 per cent glucose, 54 per cent sucrose, 2 per cent corn oil, 14 per cent hydrogenated-vegetable oil (Crisco), 2 per cent cellulose (Solka-Floc), 4 per cent salts (Jones-Foster mixture) and vitamins as described previously[5]. The added amino-acids replaced an equivalent amount of sucrose. Vitamin A was administered every other day at a level of 40 I.U. per rat.

The results of these experiments, presented in Table 2, show that the supplementation of 9 per cent casein diet with leucine and/or norleucine did not lower the storage of hepatic vitamin A as was anticipated. Instead there was a definite increase in the vitamin A content in the livers of rats receiving amino-acid supplements. The contrary observation made previously can thus be safely explained on the basis of different food intake.

The growth data are just the opposite from the vitamin A values. Norleucine markedly suppressed growth of rats especially when fed together with leucine. Supplementation of diet with leucine alone, however, resulted only in a slight growth depression. The data are conclusive in demonstrating the inverse relationship between the vitamin A content of the liver and the body weight gain.

The storage of vitamin A in the kidneys, also shown in Table 2, was found to be small when compared with the liver. It should be noted, however, that the inclusion of the excess amino-acids in the diet consistently lowered the kidney vitamin A.

Since the liver and the kidneys are the principal sites of the storage of vitamin A in the mammalian organisms, the sum of the vitamin A values in these organs, after correction for the initial content, should give the 'total' storage of vitamin A in the body. Similarly the amount of vitamin A which is lost or unaccounted for, as calculated by subtracting the vitamin A stored from the amount of vitamin A which was administered, can serve as a measure of the vitamin utilization. When such calculations are made it is evident that the more rapidly growing animals utilize more and store less vitamin A than do the slow growing animals. This hypothesis would account for the lower level of hepatic vitamin A in the rats fed casein when compared with similar diet supplemented with the excess amino-acids. This thesis is further supported by our recent observation[5] that the oral administration of equal amounts of vitamin A to growing rats resulted in a greater hepatic vitamin A storage in rats fed protein-free diet than in those which were kept on casein diet.

It is apparent from this as well as previously reported[5] studies that rats are able to store ample amount of vitamin A in the liver despite the amino-acid imbalance or in the complete absence of the dietary protein when the intake of vitamin A is controlled. Nevertheless the lack of or imbalance in the dietary protein may result in a lower storage of vitamin A simply because under these conditions the animals usually lose appetite which inevitably will result in lowered consumption of vitamin A.

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Table 1. Growth, Food Consumption and Vitamin A Storage in the White Rat Fed Imbalanced Diets

Group	No. of rats	Body weight gain (gm.)	Food intake (gm.)	Liver		Kidney		Total vit. A (μgm.)
				weight (gm.)	vit. A (μgm.)	weight (gm.)	vit. A (μgm.)	
Nitrogen-free	2	−20	96	1·25	89·1	0·38	2·8	91·8
9% Casein	4	58	229	4·13	572·6	0·74	8·6	581·2
9% Casein + 2% DL-norleucine	4	47	213	3·51	514·9	0·80	7·3	522·2
9% Casein + 2% L-leucine + 2% DL-norleucine	4	25	156	2·53	350·0	0·66	5·9	355·9

Table 2. Growth, Food Consumption and Vitamin A Storage in the White Rat Fed Imbalanced Diets under the Conditions of Equalized Intake of Vitamin A

	Control rats	Supplement to 9% Casein Diet			
		None	2% L-Leucine	2% DL-Norleucine	2% L-Leucine and 2% DL-Norleucine
Number of rats	2	3	3	3	3
Weight gain (gm.)	—	51	43	29	24
Food intake (gm.)	—	253	236	198	178
Vitamin A fed (μgm.)	—	126·0	126·0	126·0	126·0
Liver vitamin A (μgm.)	15·7	45·4	58·3	65·0	64·2
Kidney vitamin A (μgm.)	1·16	2·03	1·58	1·76	1·32
Total vitamin A stored (μgm.)	—	30·6	43·0	49·9	48·7
Vitamin A utilized (μgm.)	—	95·4	83·0	76·1	77·3

Thirst for Bile in Rats with Bile Fistulæ

SEVERAL of the trace metals are primarily excreted *via* the bile[1]. The kinetics of excretion and re-absorption of bile-bound isotopes have been the subject of a study at this Center[2,3].

Bile fistulæ were made in two operations, 24 hr. apart. In the first the common bile duct was ligated and in the second the dilated bile duct was entered with a No. 50 polyethelene catheter. The catheter was anchored *in situ*, and the bile was collected outside the cage. The effluent bile varied in amount. It was highest in some rats which weighed 250 gm. and produced about 35 ml. the first day. These animals drank all their bile with avidity within the first few minutes of their being offered the bile. Various degrees of pronounced thirst for bile have been encountered in all the bile-fistula animals so tested.

We then differentiated bile-thirst from ordinary thirst either by offering the animals water *ad libitum* or by giving them the choice between bile and water. A similar experiment was done with desiccated and reconstituted ox bile on one hand and the animals' own bile on the other; marked preference for the autogenous bile was shown by the rats. The rough impression was gained that these rats preferred their personal bile to that of another donor rat; but statistical evaluation is lacking.

A simpler operation which permitted the elimination of the second stage of this procedure caused identical thirst for bile in the operated animals. The normal animals did not care for bile.

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Effect of the Potassium Salt of Gibberellic Acid on the Growth and Reproduction Rate of *Paramecium multimicro-nucleatum*

THIS investigation was prompted by the fact that though the effects of gibberellic acid and its salts in stimulating growth of plant cells have been studied widely[1,2,3], very little is known of their effects on bacteria and other micro-organisms[4,5]. The first micro-organism chosen for this study was yeast (*Saccharomyces cerevisiae*), but its growth was found to be unchanged by potassium gibberellate ('Gibrel', supplied through the courtesy of Merck and Co., Inc.). It was then decided to study a unicellular animal, and the ciliate infusorian, *Paramecium multimicro-nucleatum*, was selected as being a simple, easily measured organism.

A culture medium consisting of 4 gm. dried foxtail in 1,000 c.c. of distilled water was prepared. This was allowed to stand uncovered for 3 days and then inoculated with *Paramecia*. After 15 days at room temperature, 10 c.c. portions of this culture were placed in test tubes containing equivalent amounts of foxtail from the original culture. To four of these tubes, 'Gibrel' was added in the proportions of 1, 10, 100 and 1,000 parts per million respectively. A fifth tube was retained as a control. Sterile cotton plugs were placed in the mouth of each tube and all were incubated at room temperature for 15 days. Slides were then prepared from each of the cultures by introducing one drop of the culture and one drop of 2 per cent formaldehyde under the cover slip. The length and width of 100 *Paramecia* from each culture were measured with the aid of a calibrated bifocal microscope.

It was found that a significant change in the size of the *Paramecia* occurred when they were treated with 100 p.p.m. of 'Gibrel' (Table 1). The other concentrations of 'Gibrel' employed caused no significant change in size.

Table 1. COMPARISON BETWEEN UNTREATED *Paramecia* AND THOSE TREATED WITH 'GIBREL' 100 PPM.

	No. Measured	Av. Length (μ)	Av. Width (μ)	Critical Ratio Length	Critical Ratio Width	Significance Level of the Increase
Untreated	100	128·0	59·0	14·29	9·96	0·1%
Treated	100	176·6	82·9			

It was noted with 100 p.p.m. of 'Gibrel' that in addition to the increased size of the *Paramecia* there appeared to be an increase in number. The experiment was therefore repeated and ten 0·5-c.c. aliquot portions of the treated and untreated cultures in clear glass dishes 1 cm. in diameter examined under low power on a bifocal microscope at 5, 10, 15, and 20 days. The number of *Paramecia* in 5 c.c. of the treated culture were found to be some 25–30 per cent higher than in the untreated culture.

It therefore appears that 100 p.p.m. of 'Gibrel' added to cultures of *Paramecia* causes a marked increase in both growth and reproduction of this unicellular animal. However, it is possible that this apparent increase in growth and reproduction is due to an increase in food supply. Work is now proceeding on the effects of 'Gibrel' on bacteria in the cultures which might act as a food source for *Paramecia*.

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A Possible Explanation for Part of the Difference in Heat Tolerance Exhibited by *Bos taurus* and *Bos indicus* Beef Cattle

USING methods based on those described by Callow[1] the carcasses of 27 head of mature boran (*Bos indicus*) cattle were analysed. When the results of these analyses are compared with those of Callow[2] for *Bos taurus* (Fig. 1) it is evident that there is a marked and progressive difference in the way in which the two species lay down fat during the fattening period, as represented by a change of from 18 to 40 per cent total separable fat in the carcass. In Fig. 1, the percentage deposition of subcutaneous and intermuscular fat of *Bos taurus* and *Bos indicus* has been plotted against the percentage total separable fat in the carcass. In the case of *Bos taurus* the difference between the levels of subcutaneous and intermuscular fat tends to decrease as fattening proceeds whilst this difference for *Bos indicus* clearly increases over the same period.

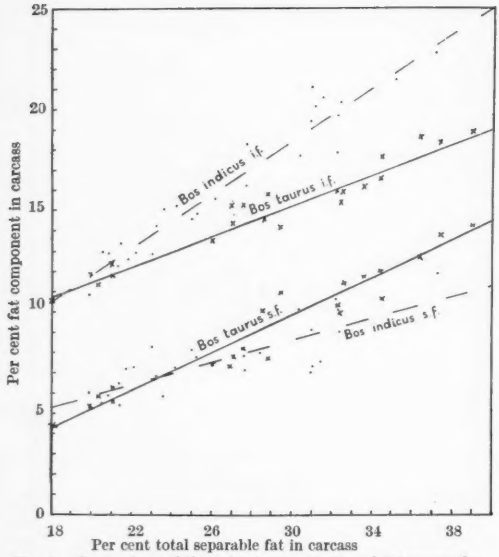

Fig. 1. Comparison of the subcutaneous (s.f.) and intermuscular (i.f.) fat deposition of *Bos taurus* and *Bos indicus*

The amount of subcutaneous fat laid down in *Bos taurus* increases during fattening from 4·4 to 14·4 per cent of the carcass—a range of 10 per cent, whilst that for *Bos indicus* increases from 5·3 to 11·3 per cent—a range of only 6 per cent.

It is suggested that these differences in the method of laying down fat may account for some of the differences in heat tolerance between *Bos taurus* and *Bos indicus* beef cattle. It is interesting to note that for carcasses containing 18–25 per cent total fat, that is from cattle killed in what in practice is referred to as the 'store' period, there is little difference between the levels of subcutaneous fat of the two species. In this connexion, it is relevant to note the oft-repeated remark in the field that 'exotic cattle don't do in the tropics'. What is meant is that they rarely advance beyond the store stage. The suggestion made here is that one of the reasons why they do not thrive is because as they increase in fatness they are genetically incapable of reducing the amount of subcutaneous fat laid down. This in turn increases heat stress and the animal is forced, possibly by reducing its intake, to return to the near store condition where it more closely resembles its zebu counterpart.

To support the simple theory that the degree of heat tolerance of cattle is partly a function of the amount of subcutaneous fatty layer might be considered to require an even greater difference between subcutaneous fat layers of the species than our results show. On the other hand, all the *Bos indicus* carcasses analysed were fattened in Kenya above the 6,000-ft. contour in a warm temperate climate. In fact the majority of the animals were grazed at Muguga, and it was apparent that they were affected by the cold. They grew thicker coats and on the colder nights stood around with arched backs. This would tend to reduce the difference between the environments to which Callow's and the Kenya cattle were subjected, and it might be expected that any differences in carcass composition that may be influenced by climatic stress would also be less.

A possible explanation as to why the two species of cattle apparently have acquired quite different methods of storing body-fat is as follows. It is reasonable to assume that subcutaneous fat in an animal acts as an insulating layer and that the degree of insulation will vary with the thickness of the subcutaneous fat. If, as is now generally assumed, the degree of heat tolerance of an animal depends upon the efficient dissipation of heat, then a thick layer of subcutaneous fat is undesirable in any animal subject to heat stress. Now if one considers the different environments in which *Bos taurus* and *Bos indicus* cattle have evolved it becomes obvious as to why they might have acquired different methods of storing fat. *Bos taurus* has evolved in an environment where there is a shortage of food but not usually of water during the colder part of the year. This has required the provision of a fat layer for re-absorption during the period of food shortage and it has also meant that some form of insulation was necessary to prevent loss of heat during the lean cold period. It would seem logical that a thick layer of subcutaneous fat would serve both these ends admirably. *Bos indicus* on the other hand has had to provide against a shortage of food during the hot season of the year when water is also scarce. In this case the animal has had to lay down fat for re-absorption, but at the same time any additional insulation preventing it from losing heat during this period would only impose an extra burden on its heat regulating mechanism. It is suggested that for this reason the species has evolved the mechanism of laying down most of its fat as intermuscular tissue.

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Carbohydrate Metabolism in the Experimental Galactose Cataract

Although the cataractogenic influence of galactose has long been appreciated[1,2,3], the mechanism whereby galactose exerts its effect on the ocular lens has not been clarified. It was recently shown that galactose-1-phosphate acts as, or gives rise to, an inhibitor of the enzyme glucose-6-phosphate dehydrogenase in the rat lens, both *in vitro* and *in vivo*[4]. In order to investigate further the hexose monophosphate shunt in such lenses, six Holtzman strain white male rats, 27 days old and weighing approximately 55 gm. each, were placed on a high galactose diet[5] while six similar animals were fed a control glucose diet[5]. One animal from each group was killed after 1½ days on its respective diet; their lenses were immediately removed, weighed, and placed in an incubation apparatus similar to the one employed by Kinoshita and Wachtl[6]. Each incubation tube contained 5 ml. Krebs Ringer bicarbonate buffer which had been gassed for 1 hr. with 5 per cent carbon dioxide, 95 per cent oxygen, in order to stabilize the *p*H at 7·5.

Two of the incubation tubes contained 50 μ moles of glucose-1-^{14}C each, with a specific activity of 2,700 c.p.m. per μ mole, while the other two tubes each contained 50 μ moles of glucose-6-^{14}C with a specific activity of 2,600 c.p.m. per μ mole. One lens from each rat was incubated with glucose-1-^{14}C while the second lens was incubated with glucose-6-^{14}C as the substrate. The gas phase employed was 5 per cent carbon dioxide and 95 per cent oxygen, and the lenses were incubated for 21 hr. at 37°C. and remained

clear during the entire incubation period. The carbon dioxide labelled with carbon-14 was precipitated as labelled barium carbonate and read in an end-window gas-flow counter. This procedure was repeated at the following time intervals: 2½, 3½, 6½, 8½ and 10½ days after the animals were placed on their respective diets.

The results of this experiment showed that in the normal rat lens most of the carbon dioxide formed from glucose was derived via the hexose monophosphate shunt. The amount of labelled carbon dioxide obtained from glucose 1-^{14}C was 15 times higher than the amount of labelled carbon dioxide derived from glucose-6-^{14}C. These results are in agreement with the data reported by Kinoshita and Wachtl[6] for the rabbit and bovine lens.

In the lenses derived from the animals maintained on the galactose diet, there was a marked reduction in the amount of labelled carbon dioxide derived from glucose-1-^{14}C. After 2½ days on the diet, the recovery of labelled carbon dioxide was 65 per cent of the normal value (from glucose-1-^{14}C) and this declined rapidly until day 8½ when the recovery was only 25 per cent of normal. However, the recovery of labelled carbon dioxide from glucose-6-^{14}C was consistently higher than the corresponding amount derived from the normal lens (about 1½–2 times).

The results of this and previous experiments[4] indicate that galactose-1-phosphate acts as, or gives rise to, a specific inhibitor of glucose metabolism in the rat lens. The inhibition of the hexose monophosphate shunt occurs at the first step of the oxidation of glucose-6-phosphate to 6-phosphogluconic acid, and this inhibition becomes apparent after 2½ days of galactose feeding. This is followed by a marked inhibition of the synthesis of soluble protein[4,7] which occurs after 6–8 days of galactose feeding. However, anærobic glycolysis not only continues unimpaired, the increased recovery of labelled carbon dioxide from glucose-6-^{14}C indicates that glycolysis may be stimulated as a compensatory mechanism following the inhibition of the hexose monophosphate shunt.

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Prophylaxis of Poisoning by Carbon Tetrachloride

CHRISTIE and Judah[1] studied the biochemical basis of the hepatotoxicity of carbon tetrachloride and found that the earliest measurable changes, which precede histological evidence of necrosis, involved disorganization of mitochondrial tricarboxylic acid cycle activity. Enzyme systems first affected were those which required pyridine nucleotides for electron transfer and inactivation of these enzymes could be largely reversed by the addition of the appropriate coenzyme. Pre-incubation of liver mitochondria from normal rats with carbon tetrachloride produced a similar pattern of inhibition[1]. It was shown that carbon tetrachloride increased mitochondrial permeability to the entry of diphosphopyridine nucleotide, and it appeared that carbon tetrachloride attacked mitochondria directly to promote the loss of respiratory cofactors, thus inactivating pyridine nucleotide-dependent enzyme systems.

The present study was undertaken to determine whether agents which stimulate the synthesis of pyridine nucleotides and thus increase tissue concentrations alter the susceptibility of rats to carbon tetrachloride poisoning.

Nicotinic acid, rather than nicotinamide, has been shown to be a precursor of pyridine nucleotides[2], as has tryptophane[3,4], which is a precursor of nicotinic acid. Administration of nicotinic acid or tryptophane stimulates the synthesis and increases the tissue concentrations of pyridine nucleotides[2,3,4].

Each of these agents was injected intra-peritoneally into rats in order to assess its effect on subsequent challenge of the rats with carbon tetrachloride administered by stomach tube. Rats of approximately 150 gm. weight were used and the dose of carbon tetrachloride given was proportional to the weight of individuals. Animals of the same sex were selected for each experiment, but no difference attributable to sex was displayed in susceptibility to or protection against carbon tetrachloride toxicity.

Table 1. EFFECT OF NICOTINIC ACID AND TRYPTOPHANE ON CARBON TETRACHLORIDE TOXICITY

Group	No. of Rats	Treatment Day 1	Day 2	Day 3	CCl_4 (ml./150 gm. rat) Day 3	Deaths
Control	52	–	–	–	0·6	52
Nicotinic Acid	17	+	+	+	0·6	2
Nicotinic Acid	8	–	+	+	0·6	5
Nicotinic Acid	18	–	–	+	0·6	16
Nicotinic Acid + glutathione	7	–	–	+	0·6	5
Control	47	–	–	–	0·4	40
Nicotinic Acid	20	–	–	+	0·4	18
Nicotinic Acid	9	+	–	–	0·4	0
Tryptophane	16	+	+	+	0·4	5
Tryptophane	9	–	+	+	0·4	6
Tryptophane	8	–	–	+	0·4	6
Tryptophane	9	+	–	–	0·4	4
Nicotinic Acid + tryptophane	9	–	–	+	0·4	9

Nicotinic acid, tryptophane and glutathione were injected intraperitoneally at the rate of 50 mgm./rat daily. Nicotinic acid was administered at doses of 25 mgm. morning and afternoon. Carbon tetrachloride was given by stomach tube.

Table 1 shows that the administration of 50 mgm./day of either nicotinic acid or DL-tryptophane for three days considerably protects against the toxicity of carbon tetrachloride given on the third day of treatment. The protective effect of nicotinic acid is the more remarkable as the dose of 0·6 ml. carbon tetrachloride/150 gm. killed all of the 52 control rats used in the several experiments. Administration of nicotinic acid or tryptophane on the day preceding and on the day of dosing with carbon tetrachloride had significant prophylactic value but less than did the three treatments. A single day's course of either compound given on the same day as carbon tetrachloride gave some but little protection, which was not increased by the administration of glutathione with nicotinic acid, reduction of the carbon tetrachloride dose to 0·4 ml./150 gm., or the combined injections of nicotinic acid and tryptophane. However, a single course of 50 mgm. of either nicotinic acid or tryptophane two days before dosing with carbon tetrachloride afforded considerable protection (Table 1).

Similar treatment with 200 mgm. methionine in 0·5 *N* hydrochloric acid by stomach tube, 50 mgm. glutathione intra-peritoneally, or 50 mgm. glucose intraperitoneally, given separately to groups of rats over three days did not affect susceptibility to carbon tetrachloride poisoning although methionine [5,6,7] and glutathione have been reported to be useful therapeutic agents.

It is clear that the administration of substances which stimulate the synthesis of pyridine nucleotides affords protection against the acute toxic effects of large doses of carbon tetrachloride. Whatever the manner by which carbon tetrachloride affects mitochondrial structure and increases permeability, this study supports the idea that it is the loss of respiratory cofactors which inactivates mitochondrial enzymes and kills cells[1]. Increased tissue concentrations of pyridine nucleotides presumably result in the maintenance of higher mitochondrial coenzyme levels and thus sustain respiratory activity in the presence of carbon tetrachloride and ensure the continuance of tissue metabolism during the critical period of maximum carbon tetrachloride effect.

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Implications of Restricting Cows to a Clover-free Diet in Order to Reduce the Strontium Content of Milk

Vose and Koontz[1] have suggested that because mixed legume-grass herbage contains a higher level of strontium than grass it would be desirable to restrict dairy cows to an all-grass diet if the level of strontium-90 in milk ever rose to a level giving cause for concern. In the same communication these authors report that the uptake of strontium by herbage is directly related to the uptake of calcium.

Since clovers usually contain substantially higher contents of magnesium, cobalt and copper than grasses, any attempt to restrict clover growth on a large scale could have very far-reaching consequences. For example, the incidence of hypomagnesæmic tetany in sheep and cattle, related as it appears to be to magnesium intake, might be seriously affected by any widespread attempt to reduce the proportion of clover in the diet of stock.

There is also the question of where the stock is to obtain the level of calcium necessary to maintain the calcium content of the milk if the intake of calcium is curtailed by reducing the clover content of the herbage. Because of the increasing tendency in the United Kingdom for farmers to adopt a policy of feeding the maximum quantity of grass to lactating cows in order to reduce the consumption of the more expensive concentrates, this is a matter of great importance. According to Corrie[2], the amount of calcium in the diet should not be less than 11–12 gm. per gallon of milk produced, and since the average milk-production ration consisting entirely of foods of vegetable origin (3½ lb.) furnishes only about 2·5 gm. calcium, it is evident that the bulk of the calcium content of milk is derived from the herbage grazed when no mineral mixture is supplied. In the case of a dairy cow yielding 5 gallons of milk per day during the summer months, the quantity of calcium supplied by a pure grass diet falls short of the lactation requirement alone, even in favourable conditions of uptake and calcium content. For example, an Ayrshire cow consuming as much as 12 kgm. of dry matter per day of grass of a relatively high calcium content, say 0·4 per cent calcium in the dry matter, will be receiving only 48 gm. calcium as compared with the 55–60 gm. required for milk production alone. Under such conditions one would expect the skeletal reserves of calcium to be rapidly depleted.

Table 1. Strontium, Calcium and Magnesium Contents of Forage Plants on a Dry-Matter Basis

Sample	Sr (p.p.m.)	Ca %	Mg %	Sr/Ca ×10⁵
Brussels sprout leaves	47·1	2·50	0·182	188
Cauliflower leaves	54·0	3·26	0·179	165
Broccoli leaves	27·9	1·64	0·117	170
Beet pulp	23·6	0·555	0·177	425
Turnip cores	12·7	0·518	0·104	245
Turnip cores	18·9	0·511	0·193	370
Clover—variety Kersey	36·6	2·15	0·234	170
Clover—variety Kersey	36·5	2·55	0·241	143
Perennial rye-grass + wild white clover	19·4	1·79	0·160	108
Perennial rye-grass + wild white clover	18·9	1·28	0·143	148
Perennial rye-grass	10·9	0·414	0·119	263
Perennial rye-grass	7·59	0·347	0·096	219

While some feeding stuffs other than clovers could supply additional calcium and magnesium, it appears unlikely that they could do so without a concomitant increase in strontium content. The following figures for the contents of strontium, calcium and magnesium in various kinds of fodder, illustrate this difficulty. Calcium was determined by a modification of the Lundegardh technique described by Mitchell[3] in which phosphate in the plant extracts was removed by ion-exchange prior to excitation in the air-acetylene flame. Strontium and magnesium were determined by the method of Farmer[4].

The leaves of brassicas generally contain even higher concentrations of calcium than those of legumes and, as the figures in Table 1 suggest, these are related to correspondingly higher contents of strontium. Of the samples analysed, those with the most unfavourable ratios of strontium to calcium were the roots and the samples of pure perennial ryegrass.

Since brassicas are directly consumed by humans and form a substantial proportion of their diet the amounts of strontium ingested in such foods may well be comparable to the amounts obtained from milk. It is clear that any general attempt to reduce the intake of strontium-90 by humans, either by selecting foodstuffs containing low contents of strontium, or by restricting dairy cows to an all-grass diet would have very serious repercussions throughout agricultural industry as well as producing dietary and nutritional problems the implications of which do not seem to have been appreciated.

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Induced Tolerance to D-Tubocurarine Chloride in the Cat

In the course of experimental work we found it necessary to administer a standard dose of D-tubocurarine chloride to the same cat on several occasions over a period of months. We observed that in the fifteen animals studied the duration of paralysis of respiratory and other muscle groups became steadily shorter. The work was all done on the intact animal in a warm room. Nitrous oxide in 3 : 1 mixture with oxygen was the sole anæsthetic agent used—thus, in the preliminary stages no drug was involved which was likely to influence the duration of neuromuscular block. D-tubocurarine was administered intravenously in doses of 0·6 mgm./kgm., and during the period of respiratory paralysis artificial respiration was maintained through an endotracheal tube at a rate of 20–25 per min., a soda lime canister being included in the circuit for the absorption of carbon dioxide. In this way adequate oxygenation was maintained throughout all experiments.

So as to investigate further the apparent change in tolerance to D-tubocurarine we compared the duration of paralysis in the anterior tibialis muscle in a group of six animals which were receiving the drug for the first time with four others which had been rendered tolerant by its repeated administration. The painful stimuli involved in setting up a nerve-muscle preparation are such that deeper anæsthesia rather than that provided by nitrous oxide is required. Thiopentone sodium was selected as an extra agent on the grounds that the level of dosage is readily controlled and it is rapidly eliminated from the blood stream. This drug was administered both to experimental and control animals. Anæsthetic induction was therefore carried out with thiopentone sodium, the initial dose of which was 20–50 mgm. Further doses were given as required during the preparatory phase; but a total of 200 mgm. was never exceeded. Endotracheal intubation was performed, and an interval of at least 30 min. allowed to elapse between the last administration of thiopentone sodium and the intravenous injection of D-tubocurarine in a dose of 0·6 mgm./kgm. body-weight. From this time until the end of the experiment, anæsthesia was maintained with a mixture of nitrous oxide and oxygen (3 : 1).

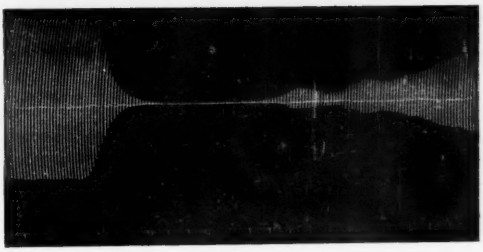
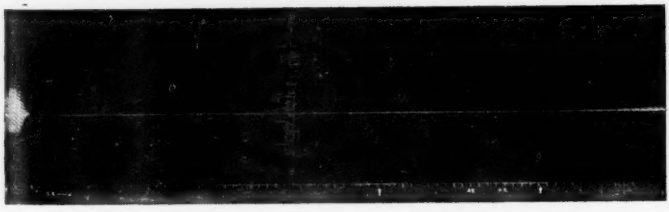


Fig. 2. Myograph of cat No. 14, in which 'tolerance' had been induced. *A* again indicates the injection of D-tubocurarine (0·6 mgm./kgm. body-weight); note that muscle contractions never disappear completely

A Brown-Schuster myograph was used to record the contractions of the anterior tibialis muscle. In order to mimimize temperature drop the limb was mounted horizontally[1], the skin was sutured over all incisions, and the laboratory was maintained at a temperature of 68° F. The general environmental conditions were therefore identical to within a few degrees in both groups. Maximal contractions were induced at 4-sec. intervals, through a platinum electrode, and stimulation was continued throughout. The periods elapsing between the administration of D-tubocurarine and the return of muscle contractions, in the four cats in which an altered response to the drug had been induced, are compared with similar periods elapsing in the six cats which had never previously received the drug. The difference between the mean duration of paralysis (20 min.) in the two groups is significant at the 2 per cent level (Table 1).

Thus we feel that our original observation has been confirmed, and that this is a genuine example of induced drug tolerance. Myograph tracings taken from 'normal' and 'intolerant' animals are shown in Figs. 1 and 2.

The mechanism of this altered response is obscure and is being further examined.



↑ *A* ↑ *B*

Fig. 1. Myogsaph of a 'normal' cat, No. 18. *A* indicates intravenous injection of D-turbocurarine (0·6 mgm./kgm. body-weight), and *B* the first perceptable muscle contraction. Time marker, 30-sec. intervals

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Table 1. Duration of Paralysis of Anterior Tibialis Muscle in the Cat due to D-Tubocurarine Chloride (0·6 mgm./kgm. Body-Weight)

Animals receiving the drug for the first time			Animals rendered tolerant by repeated administration of the drug				
No. of animal	Body-weight (kgm.)	Duration of paralysis (min.)	No. of animal	Body-weight (kgm.)	Duration of paralysis (min.)	No. of previous injections	Period over which drug previously give (weeks)
C 18	3·2	30	*E* 5	2·7	4	23	74
C 19	2·7	44	*E* 12	2·3	11	10	6
C 20	2·3	31	*E* 13	2·7	11	9	18
C 21	2·6	11	*E* 14	1·5	0	10	13
C 22	2·4	18					
C 23	1·7	25					
Mean		26·5000			6·50000		
Variance		108·9167			22·2500		

t, 3·2161. n, 8. $P < 0·02$

Maintenance of Pregnancy in Ovariectomized Ewes by Means of Progesterone

Ovariectomy may be performed in the ewe as early as the 50th day of pregnancy without resulting in abortion[1]. It has been assumed that by this stage the steroid hormones produced from extra-ovarian sources are sufficient to maintain pregnancy. This supposition is supported by the fact that progesterone has been found in the placentæ of ewes 100 days pregnant[2], and also in the adrenal venous blood of both gonadectomized rams and ewes[3]. However, little is known of the steroid hormonal requirements of the ewe during pregnancy. Foote *et al.*[4] treated ewes, ovariectomized 3½ days after mating, with either progesterone alone or progesterone together with œstrone, and found at slaughter 25 days after mating, that almost 50 per cent of the ewes contained normal embryos.

In the course of a recent study involving egg transfer in the ewe, fertilized sheep eggs at the 8–16 cell stage were collected from donor ewes and transferred to the uterine horns of 36 recipient ewes. Both the donors and recipients were of the Welsh Mountain breed. Twenty-four of the recipients were ovariectomized at the time of transfer, 3–4 days after œstrus, and then treated with either progesterone alone at doses of 10 or 20 mgm./day, or with similar amounts of progesterone together with 5 μgm. œstradiol benzoate. The remaining 12 recipients were left entire and acted as control animals (Table 1). Of each group of 12 ewes,

Table 1

Treatment	No. of ewes	Total No. of eggs transferred	No. of Ewes lambing	No. of lambs born
Spayed Ewes				
Progesterone alone	12	42	10	17
Progesterone + œstradiol benzoate	12	42	6	8
Control Ewes				
None	12	42	10	19

6 received 5 eggs and 6,2 eggs, making a total of 42 eggs transferred to the animals in each of the 3 groups. Treatment of the spayed ewes was discontinued on the 60th day after œstrus and both control and spayed ewes were allowed to go to term.

Sixteen (67 per cent) of the 24 spayed ewes and 10 (83 per cent) of the 12 control animals afterwards lambed, producing 25 and 19 lambs respectively (Table 1). Of the 16 spayed ewes which lambed, 10 had been treated with progesterone alone and 6 with progesterone plus œstradiol benzoate. Significantly, more of the eggs transferred to ewes treated with progesterone alone developed into lambs than of those transferred to ewes treated with both hormones ($\chi^2 = 4{\cdot}61$; $P < 0{\cdot}05$). Neither the number of ewes which lambed nor the number of lambs born to the spayed ewes treated with progesterone alone differed significantly from control ewes, but significantly fewer of the eggs transferred to the spayed ewes treated with progesterone and œstradiol benzoate developed into lambs than in the control ewes ($\chi^2 = 6{\cdot}60$; $P < 0{\cdot}02$). The actual dose of progesterone administered to spayed ewes, irrespective of whether given alone or with œstradiol benzoate, had no effect upon the lambing results; of the 16 spayed ewes which lambed 8 had been treated with 10 mgm./day and 8 with 20 mgm./day progesterone. No abortions were observed in the spayed ewes after the cessation of treatment and the mean length of gestation of both control and spayed ewes was 147 days. Parturition in the spayed ewes was quite normal and the mean birth-weight of lambs born to these animals did not differ significantly from that of the control ewes.

It would seem that progesterone alone in daily dose levels of 10 or 20 mgm. is capable of maintaining pregnancy in ovariectomized ewes up to the 60th day of pregnancy. The presence of the ovaries does not seem to have any great influence on determining the length of gestation, nor do they appear to be essential for the processes of parturition.

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Effect of Vitamin D on the Respiration of Rat Oral Tissue

Little is known of the metabolic changes occurring in mammalian oral tissues. Glickman[1] determined the oxygen quotient, $Q(O_2)$, (μl. oxygen taken up per mgm. dry tissue per hr.) of normal human gingiva and found it to be $1{\cdot}6 \pm 0{\cdot}37$. In 1950[2] he reported that the $Q(O_2)$ of normal dog gingiva was $1{\cdot}3 \pm 0{\cdot}07$. Recently we have shown that a complete citric acid cycle exists in sheep gingiva, the $Q(O_2)$ for this tissue being[3] $1{\cdot}46 \pm 0{\cdot}33$. This work has been extended to include rat oral tissues in which the occurrence of a citric acid cycle has also been demonstrated[4]. The present report is concerned with the effect of vitamin D on the metabolism of rat palatal mucosa.

The tissues were taken from both normal and rachitic rats of the Wistar Institute strain. Histological examination confirmed that only the epithelium and a little sub-epithelian connective tissue was removed. Normal rats, fed on the stock diet used in this department, were taken for study when weighing about 60–80 gm. Another group was placed when weighing about 60 gm. on a modified Sherman-Pappenheimer rachitogenic[5] diet for 21 days. The diagnosis of rickets was made on radiological examination and after staining the epiphyses of the radii and ulnæ with silver nitrate[6]. Water-soluble vitamin D was prepared according to the method of Zetterström[7] and was found to be biologically active when given to rachitic rats. The $Q(O_2)$ of the palatal mucosa was determined by the direct method of Warburg, the procedure and solutions being as described by Umbreit *et al.*[8] Only a 30 per cent change in the $Q(O_2)$ as compared with controls was taken as being significant.

The basal $Q(O_2)$ values of normal and rachitic tissues showed no significant differences (Table 1). With both tissues the addition of succinate or malate produced a rise in oxygen consumption. When soluble vitamin D was added to the reaction mixture it had no effect upon the basal oxygen uptake of either type of tissue, nor upon the oxygen consumption of normal tissue in the presence of the two metabolites. However, in the case of mucosa from rachitic rats, vitamin D produced a further rise in oxygen consumption when succinate or malate was present (Table 1). The above effects were also noted when pyruvate or citrate

Table 1. $Q(O_2)$ VALUES OF FRESH NORMAL AND RACHITIC RAT PALATAL MUCOSA

	No substrate added	Phosphorylated vitamin D added	Succinate added	Succinate + phosphorylated vitamin D added	Malate added	Malate + phosphorylated vitamin D added
Normal rat mucosa	1·96 ± 0·03 (35)	2·13 ± 0·016 (18)	3·34 (3)	3·53 (3)	1·94 (3)	2·29 (3)
Rachitic rat mucosa	1·92 ± 0·02 (17)	1·94 ± 0·018 (7)	2·65 (3)	3·92 (3)	2·53 (3)	4·49 (3)

1 ml. of $M/50$ solutions of various intermediates of the Krebs citric acid cycle were added to the reaction flasks in a total volume of 2·55 ml. 0·05 ml aqueous 0·5 per cent phosphorylated vitamin D solution was introduced where indicated. Figures in brackets indicate number of observations. Where only three observations were made the mean value is given and the t test was applied to the results which were found to be significant at the 5 per cent level for succinate and the 3 per cent level for malate in the case of rachitic tissue.

was used. This action of vitamin D was found only in fresh tissue, since after storage for one day at 4° C. no such effect on oxygen uptake could be detected although the metabolites still increased the oxygen consumption above the basal value.

Zetterström[9] has described an activation of aerobic oxidation in kidney mitochondria by phosphorylated vitamin D using glutamic acid as oxidizable substrate. De Luca *et al.*[10], found that vitamin D suppressed citrate oxidation in kidney mitochondria of normal and rachitic rats, but no effect of vitamin D on citrate oxidation was observed in liver mitochondria of these animals.

The above observations seem to be at variance as to the precise mode of action of vitamin D in metabolic processes. Our results would suggest that this vitamin may play an important part in the direct regulation of the oxidative phase of carbohydrate metabolism. However, the possibility must be considered that vitamin D is exerting its influence indirectly by affecting the permeability of the metabolites of the citric acid cycle through the cell wall or mitochondrial membrane.

Further work is in progress to elucidate this mechanism.

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Blood Acetylcholinesterase in *Phalaris* Staggers

RUMINANTS grazing pastures containing a large proportion of growing *Phalaris tuberosa* on occasions develop a nervous disorder, phalaris staggers[1,2], the symptoms of which are an initial hyperexcitability followed by muscular tremors, unco-ordinated gait and stumbling or falling when driven. Demyelination in the spinal cord ensues and complete recovery has not been observed. On the grounds that similar symptoms and pathological changes occur in cats[3], dogs[4] and hens[5] treated with cholinesterase inhibitors, Dr. J. H. Gaddum (personal communication to Dr. H. R. Marston) suggested that some knowledge of the cholinesterase activity in the blood of animals suffering from phalaris staggers might illuminate the origin of the disorder.

Blood was collected into heparinized tubes from the jugular veins of sheep that had recently developed visible symptoms of the malady and which were still grazing on *P. tuberosa*. Control samples of blood were collected from animals grazing the same pasture but which were protected from staggers by prior administration of cobalt pellets[6]. Further controls were obtained from penned animals fed on a wheaten hay chaff-gluten diet.

Acetylcholinesterase was measured in whole blood by Ammon's[7] method after dilution 1 in 5 with distilled water to dilute the enzyme suitably and to hæmolyse the red cells.

The results obtained (Table 1) show that no significant difference exists between the blood acetylcholinesterase activities in normal and staggering sheep. These findings support results obtained by Lee and Good (unpublished) who demonstrated that the blood cholinesterase level of sheep could be reduced to a very low value by feeding the powerful anticholinesterase ethylthioglycol diethyl thiophosphate (Systox) without producing symptoms of nervous debility.

Table 1. ACETYLCHOLINESTERASE ACTIVITY OF BLOOD OF NORMAL AND STAGGERING SHEEP

	Normal	Staggering
Number sampled	12	12
Range of values obtained*	35–56	33–62
Mean value	48·2500	45·1667
Standard deviation	3·3844	
't' value $\left\{\frac{\text{difference of the means}}{\text{standard deviation}}\right\}$	0·91 (not significant)	

* Activity expressed as μmoles acetylcholine hydrolysed/hr./ml. blood.

My thanks are due to Dr. H. R. Marston for introducing me to this problem and to Messrs. H. J. Lee, R. E. Kuchel and R. F. Trowbridge for collection of samples in the field. Dr. E. A. Cornish of the Division of Mathematical Statistics was responsible for statistical interpretation of the data.

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Effect of Acetylcholine on the Incorporation of Radiophosphorus into the Phosphatido-Peptide Fraction of Pancreas Slices

EARLIER work by Hokin[1] has shown that pancreas slices actively secrete the digestive enzymes, amylase, lipase and ribonuclease, when incubated in the presence of acetylchlione and eserine. Hokin and Hokin[2,3] have shown further that the stimulation of enzyme secretion was associated with a marked stimulation of the incorporation of radiophosphorus into the phospholipide fraction of pancreas slices. With acetylcholine, they found that the incorporation of radiophosphorus was increased approximately 1,000 per cent in the non-choline-containing phospholipides.

Huggins and Cohn[4] have recently reported the isolation of a phosphatido-peptide fraction from the renal cortex of several species. The fraction was also found in brain, heart, liver, lung, spleen, pancreas, intestine and Ehrlich ascites tumour cells. The phosphatido-peptide, which is believed to be a distinct phosphorus-containing fraction, appeared to consist of inositol, phosphate, esterified fatty acids and amino-acids, presumably in peptide linkage. Studies with radiophosphorus have shown that the specific activity of the phosphatido-peptide fraction was second only to the acid-soluble phosphorus fraction and was some 10–15 times that of the phospholipide and nucleic acid phosphorus fractions.

The reported observation that the phosphatido-peptide fraction has a relative high specific activity[4] and the report by Huggins, Burford and Cohn[5] that phosphoinositide is present in the phosphatido-peptide fraction suggested that the incorporation of phosphorus into such a fraction might be increased in slices of pancreas tissue during stimulation of their secretory activity.

Slices of pancreas were prepared from chickens fed *ad libitum* until they were killed. The slices were incubated in Warburg flasks containing medium III of Krebs[6] at 40°C. using oxygen as the gas phase. The incubation medium contained varying concentrations of acetylcholine, 3×10^{-4} *M* physostigmine salicylate (eserine) and approximately 30 μc. of disodium hydrogen phosphate labelled with phosphorus-32. After incubation for two hours the slices were removed and separated into the different phosphorus-containing fractions as described by Huggins and Cohn[4]. The incubation medium was stored at −20°C. until assayed for amylase content by the method of Bernfeld[7].

The results presented in Table 1 show that maximal stimulation of the turnover of phosphorus into the phosphatido-peptide fraction occurred in the presence of 10^{-4} *M* acetylcholine. At this concentration there was an increase of more than 500 per cent in the specific activity of the phosphatido-peptide phosphorus. Similar results were obtained with the phospholipide fraction. Maximal secretion of amylase by the slice occurred in the presence of 10^{-6} to 10^{-5} *M* acetylcholine. Atropine sulphate, 10^{-4} *M*, when added to the incubation medium containing acetylcholine, inhibited the stimulatory effect of the latter by 75 per cent. The results presented here suggest that phosphatido-peptide as well as phospholipide may play a part in the secretory activity of the pancreas.

Hokin and Hokin[8] have reported that they obtained two separate phosphoinositides in their phospholipide fraction if they include an acidified organic solvent in the extraction procedure. They report that both of these phosphoinositides were stimulated to incorporate radiophosphorus when acetylcholine was added to pancreas slices under *in vitro* conditions. It is possible that one of these phosphoinositides is similar to or identical with the phosphoinositide of the phosphatido-peptide fraction since the latter is obtained from tissues by extraction with acidified chloroform-methanol after removal of the phospholipide fraction by extraction with neutral chloroform-methanol. These relationships are under active investigation as well as the possibility that the phosphatido-peptide may be a part of the active portion of the membrane involved in secretory mechanisms.

Table 1. THE EFFECT OF INCREASING CONCENTRATIONS OF ACETYLCHOLINE ON THE INCORPORATION OF RADIOPHOSPHORUS INTO PHOSPHORUS-CONTAINING FRACTIONS IN SLICES OF CHICKEN PANCREAS

Concentration of acetylcholine	Other additions	Specific activity*		
		Phosphatido-peptide	Phospholipide	Organic acid-soluble
M	*M*	c.p.m./μgm. phosphorus	c.p.m./μgm. phosphorus	c.p.m./μgm. phosphorus
0	0	1399	314	11,230
10^{-7}	eserine 3×10^{-4}	1680	548	11,965
10^{-6}	"	4005	845	13,165
10^{-5}	"	4145	930	15,725
10^{-4}	"	7540	1017	11,590
10^{-3}	"	6480	888	12,670
10^{-2}	"	4705	767	10,300

* The specific activity data are calculated to 100,000 c.p.m. per μgm. phosphorus in the incubation flask.

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Presence of Certain Onium Compounds in Brain Tissue following Intravenous Administration to Rats

SOME of the effects produced in the intact animal by a number of quaternary ammonium compounds, such as D-tubocurarine chloride in high doses[1], tetraethylammonium chloride and hexamethonium[2,3] following intravenous administration have been attributed to the action of these agents on the central nervous system. Conclusions concerning the possibility of these agents acting centrally as well as peripherally, however, can be only speculative since there is little convincing actual evidence that any of the positively charged organic compounds can reach the tissues of the central nervous system following peripheral administration. The presence of prostigmine in the effluent from perfused cerebral ventricles of cats following intravenous administration has been demonstrated by indirect bioassays[4]. During the course of an investigation of the physiological disposition of hexamethonium and some of its derivatives[5], the sulphonium analogue, hexamethylene *bis*dimethylsulphonium dibromide, was detected in brain tissue of rats following parenteral administration.

This finding prompted further study of the distribution between blood and brain of this and other onium compounds.

The compounds used in this study were: hexamethonium (C_6—hexamethylene bistrimethylammonium dibromide); hexamethylene *bis*dimethylsulphonium dibromide; hexamethylene *bis*diethylsulphonium diiodide and an unsymmetrical bisquaternary, N-(6-trimethylaminohexyl)-*iso*quinoline dibromide.

The compound under study was injected via the saphenous vein into an unanæsthetized Sprague-Dawley rat (weight about 200 gm.). After an appropriate interval the animal was killed and bled before removal of the brain tissue. The brain tissue was quickly wiped free of any surface blood, and macroscopically visible blood vessels carefully removed. The method of preparation of tissues for chemical analysis has been previously described[6]. The amount of the compound in blood and brain tissue was determined by using modifications of the bromphenol blue method used to demonstrate the penetration of acetylcholine into viable squid axons[7,8]. These modifications involved changes only in the sample size and the amount of alkaline salts used in the colour-reaction flasks. For hexamethonium a 7 ml. aliquot of the metaphosphoric acid filtrate was added to a flask containing 3·0 gm. sodium carbonate (anhydrous) and 0·6 gm. dipotassium hydrogen phosphate (anhydrous); for hexamethylene *bis*dimethylsulphonium bromide and hexamethylene *bis*diethylsulphonium diiodide the aliquot was 4 ml. and the salts 1·5 gm. sodium carbonate and 0·6 gm. dipotassium hydrogen phosphate for N-(6-trimethylaminophenyl)-*iso*quinoline dibromide the aliquot was 8 ml. and the salt 3·0 gm. sodium carbonate[5]. The bromphenol blue method is specific for onium compounds in which at least one chain is 4 carbons or longer and there is no reading at 600 mμ for tissue or reagent blanks with either the original method or its modifications. The sensitivity of the method permits determination of as little as 1–2 μgm. of the onium compounds per gram of tissue with an accuracy of ±0·5 μgm.

Table 1. Distribution of Onium Compounds Between Blood and Brain Following Intravenous Administration to Rats

Compound	Time* Minutes	No. of Rats	Blood Concn. μgm./ml. Mean	Blood Concn. μgm./ml. Range	Ratio: Brain μgm./gm. Blood μgm./ml. Mean	Ratio: Brain μgm./gm. Blood μgm./ml. Range
DMS	*30*	*2*	*18*	*18, 18*	*0·39*	*0·33–0·44*
DMS	60	7	15	12–18	0·64	0·35–0·92
DMS	90	2	11	10–12	0·95	0·89–1·0
DES	40–60†	4	83	52–108	0·15	0·08–0·19
IN 181	*30*	*2*	*4*	*3–5*	*0·53*	*0·40–0·67*
IN 181	60	2	2	2, 2	1·0	1·0, 1·0
C_6	30	2	85	70–100	0·19	0·12–0·25
C_6	60	3	66	30–106	0·13	0·06–0·23

* Elapsed time from start of injection to sacrifice.
† Animals died at times ranging from 40 to 60 min.
DMS, hexamethylene bisdimethylsulphonium dibromide;
DES, hexamethylene *bis*diethylsulphonium diiodide;
IN 181, N-(6-trimethylaminohexyl)-*iso*quinoline dibromide.

Table 1 is a summary of the results and shows the distribution between blood and brain at various times after the administration of each of the four onium compounds. Following the dose of 30 mgm./kgm. the dimethylsulphonium derivative was found in the brain tissue within 30 min., and the ratio of its concentration in blood and brain approached unity between 60 and 90 min. The diethylsulphonium derivative could not be detected in brain tissue until the intravenous dose was raised to 75 mgm./kgm. At this dose-level the animals died within 40–60 min., death being preceded by clonic convulsive activity. In order to obtain a non-toxic level at which C_6 could be detected in brain, the total dose was also increased to 75 mgm./kgm., but given in two injections spaced 6 min. apart. N-(6-trimethylaminophenyl)-*iso*quinoline dibromide was found in brain following doses of only 15 mgm./kgm. At the doses used, we failed to obtain evidence of distribution to the brain at times earlier than those indicated in Table 1; but this does not exclude the possibility that these agents are present in brain tissues soon after administration in amounts too small to be detected by the methods used.

Since the chemical method used for determination is specific for onium compounds and does not have to be corrected for tissue or reagent blanks, even values at the lower limit of sensitivity represent real onium content in the tissue. In each case the actual amounts determined in brain tissue were well in excess of any artefact due to blood content of brain since exsanguinated rat brain contains on the average only 0·018 ml. of blood per gram[9]. Although there is no question that each compound measured in brain tissue is an onium cation, the small quantities present preclude their absolute identification as the original, unchanged compound. However, in other studies, both C_6[5,10] and hexamethylene *bis*dimethylsulphonium bromide[5] were found to undergo no biotransformation in the intact animal. The specificity of the chemical method is such that minor changes in either hexamethylene *bis*diethylsulphonium diiodide or N-(6-trimethylaminophenyl)-*iso*quinoline dibromide might go undetected, but these changes could not involve the substituent groups on the positively charged atom and, therefore, the compounds transferred from the blood to the brain would still be onium cations.

The results of these preliminary distribution studies make quite tenable the conclusions that the hypotensive effects of C_6 may be mediated through both a central and a peripheral site of action[2,3] and also that some of the actions of other onium compounds may be attributed to their action on the central nervous system. The presence of a positive charge on the onium compounds does not of itself prevent passage of these agents across the 'blood-brain barrier'.

There have been attempts to correlate the rates of penetration of a number of drugs into the central nervous system with their lipoid solubility, measured in various ways[11]. However, when we measured lipoid solubility by these conventional methods, we could not explain the passage of the above onium compounds into the brain since we found them to be much less 'lipid-soluble' than some monoquaternary ammonium compounds (for example, oxyphenonium) which did not gain access to brain tissue in measurable amounts during constant perfusion.

A full report of these and related studies is in preparation and will be published elsewhere.

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Influence of Sexual Stimulation on the Metabolic Activity of Deoxyribonucleic Acid in the Seminal Vesicle

LABELLED adenine[1] and thymidine labelled with tritium[2] are incorporated into the deoxyribonucleic acid of the epithelial cells of the seminal vesicle of adult mice at a rate equivalent to a renewal of approximately 10 per cent of the deoxyribonucleic acid per day. Comparison of counts of cells in division and labelled nuclei in autoradiographs of the seminal vesicle and œsophagus[2] show that this renewal of deoxyribonucleic acid is approximately 45 times in excess of requirements for cell-proliferation. It seemed of interest to explore whether this incorporation of thymidine is influenced by the sexual activity of the animals.

Six male mice of an inbred strain, 6–8 weeks old, were kept in cages together with an equal number of females whilst a second group of six males were separated from females. Eight days later all animals were injected intraperitoneally with 20 μc. each of labelled thymidine and killed 19 hr. later. The seminal vesicles were fixed for 1 hr. in acetic acid—alcohol (1:3) followed by 24 hr. in formol saline, embedded in paraffin-wax and sectioned at 5–6 μ. Autoradiographs were prepared by the stripping film technique, exposed for 40 days and stained with hæmatoxylin after photographic processing.

Analysis of the preparations shows incorporation of labelled thymidine in epithelial nuclei of the seminal vesicles of all males kept together with females; the percentage of labelled nuclei and strength of autoradiographs are similar to those reported previously[2]. No labelled differentiated epithelial nuclei were found in the organs of males which had been separated from females for eight days. A very low incidence of labelled basal-layer nuclei was noted in both groups.

Previous observations on altogether 34 male mice which had been kept together with females showed incorporation of labelled thymidine in the seminal vesicles whilst those of a group of 10 animals which were accidentally separated from females one week before injection showed none. Similarly, Schooley[3] Kelly and did not find any labelled nuclei in the epithelium of the seminal vesicles of virgin male mice after injection with labelled thymidine.

It was suggested in previous papers[1,2] that the observed incorporation of labelled precursors into deoxyribonucleic acid in excess of requirements for cell-division indicates metabolic activity of the deoxyribonucleic acid in the organs concerned, though no direct connexion between these processes could be shown. The observations reported here indicate such a connexion between biological activity and incorporation; they do not make it certain whether the incorporation of labelled thymidine into the deoxyribonucleic acid of the seminal vesicles is connected with copulation or merely with stimulation due to the presence of females. It is known that the amount of cell-division in the seminal vesicle depends on the concentration of sex-hormones. Since, however, the excess of labelling over mitotic activity was found by comparing counts on adjacent sections of the same animals[2], increase in cell-division cannot be responsible for the differences in uptake of labelled thymidine due to sexual activity reported in this communication.

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Chemistry and Pharmacology of 16-Acetyl Digitalinum Verum

THE 16-acetyl derivative of digitalinum has been prefaced as follows. Digitalinum verum hexaacetate was obtained by the usual reaction of digitalinum verum with acetic anhydride in pyridine solution; this hexaacetate, using snail enzyme[1], was partially deacetylated by potassium hydrogen carbonate to digitalinum verum diacetate, m.p. 181°–184°, $(\alpha)_D$ −24·0° (CH_3OH). The substance was separated by partition chromatography from the reaction mixtures, but it could not be induced to crystallize. The existence of acetoxyl group on carbon atom 16 of this glycoside has been confirmed by the facts that on treatment with alumina it affords a hydrogenated derivative, and that the molecular rotation difference between digitalinum verum and this compound is similar to the difference between gitoxigenin and oleandrigenin. Evidence is also given by the fact that it is hydrolysed into oleandrigenin on Mannich hydrolysis at low temperature (0°–5°).

The pharmacological action of 16-acetyl digitalinum verum was compared with digitalinum verum or *k*-strophanthin using various animals such as frogs, guinea pigs and cats. First, cardiotonic action was investigated. Drugs were administered to the isolated frog heart through which Ringer's solution was perfused. 16-Acetyl digitalinum verum augmented the amplitude of ventricular contraction and increased the output in concentrations above 1:7,500,000. Digitalinum verum and *k*-strophanthin acted similarly in concentrations above 1:5,000,000 and 1:10,000,000 respectively. Guinea pig heart-lung preparations were also used. 16-Acetyl digitalinum verum increased the output and arterial pressure and decreased right intra-atrial pressure in concentrations above 1:10,000,000 and *k*-strophanthin acted similarly above 1:20,000,000. From these results, 16-acetyl digitalinum verum seemed to be weaker than *k*-strophanthin but stronger than digitalinum verum in cardiotonic action.

Secondly, lethal doses were measured. In frogs, LD_{50} was measured by injecting drugs subcutaneously. In guinea pigs and cats, minimal lethal doses

Table 1. TOXICITIES OF VARIOUS CARDIAC GLYCOSIDES

		16-Acetyl digitalinum verum (mgm./kgm.)	Digitalinum verum (mgm./kgm.)	*k*-Strophanthin (mgm./kgm.)
LD_{50} in frogs		0·49 (25)	1·38 (25)	0·44 (25)
MLD in cats		0·43 (3)	1·61 (4)	0·17 (2)
MLD in guinea pigs	Intervals of injection (min.)			
	5	0·90 (3)	16·1 (3)	0·53 (5)
	10	1·47 (3)	22·3 (3)	0·52 (5)
	20	2·50 (3)	40·0 (3)	0·29 (5)

Number of animals used in brackets.

were so measured that about one tenth of minimal doses, which had killed animals intravenously in preliminary experiments, was injected intravenously every 5 min. until death occurred. As shown in Table 1, 16-acetyl digitalinum verum seemed to be weaker than *k*-strophanthin but much stronger than digitalinum verum. Moreover, in guinea pigs, minimal lethal doses were measured using the same procedure as before except that injections were given every 10 or 20 min. As shown in Table 1, for 16-acetyl digitalinum verum and digitalinum verum, doses in cases of 10 or 20 min. were required much more so than those of every 5 min.; however, for *k*-strophanthin, the dose in the case of every 10-min. injection was almost the same as that of every 5 min. and more than that of every 20 min. Therefore, 16-acetyl digitalinum verum and digitalinum verum seemed to be more speedy than *k*-strophanthin in rates of action and elimination.

It has been reported that some natural glycosides which consist of oleandrigenin (16-acetyl gitoxigenin) as aglycone are more potent than their 16-hydroxy glycosides derived by artificial deacetylation. But it is the first time that the acetyl group to 16 hydroxy of gitoxigenin glycoside containing glucose in sugar moiety has been introduced. With the preparation of this substance we felt that we had accomplished our purpose of synthesizing from gitoxigenin glycoside a glycoside with activity stronger than that of the parent compound.

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Changes in Purine Nucleotides of Red Lateral Muscle of Rainbow Trout

IN earlier papers[1,2] we have reported studies on the changes in acid-soluble nucleotides of carp muscle in which they found the accumulation of inosinic acid as a result of slow freezing. Further, the changes of these compounds with time in red lateral and in dorsal muscle of rainbow trout were followed by using ion-exchange chromatography. It was found that the enzyme activities of the former affecting the breakdown of nucleotides are greater than of the latter.

Living fish, scooped with a net, were decapitated, skinned and filleted immediately. The fillets were stored at an ambient temperature of 0° C. for several days. Two gm. of muscle which were immediately desected from the red lateral or dorsal parts of the fillet were ground by hand in a mortar in 20 ml. of 4 per cent cold perchloric acid solution. The extract was filtered and 10 ml. of the filtrate were used for analysis.

Nucleotides and nucleosides were separated by the hydrochloric acid gradient elution system on 'Amberlite *IRA*-400'. The details of the system were described previously[2].

In Table 1 the concentrations of the compounds present after various time-intervals are shown. From these results two characteristics in red lateral muscle, as compared with dorsal one, are noticeable.

The first is the difference in the total amounts of purine compounds, especially those of nucleotides. Value (μ moles/gm. of muscle wet-weight) for the former is smaller than for the latter. This is also supported by other values which in mackerel and in trout muscle are 6·47–7·80 and 4·68 for red lateral, 9·30–10·73 and 7·53 for dorsal muscle (T. Saito, K. Arai, T. Yajima, unpublished work).

Table 1. THE CHANGES OF THE PURINE COMPOUNDS OF RAINBOW TROUT MUSCLE

	Compound found \ Time (hr.) elapsed	0	3	6	24	72
Dorsal muscle	Hypoxanthine			0·14	0·23	0·58
	Inosine	0·00	0·29	0·63	1·02	1·58
	Adrenosine monophosphate	0·42	0·17	0·14	0·17	0·20
	Inosinic acid	3·36	4·23	6·30	6·09	5·42
	Adenosine diphosphate	1·04	1·11	0·30	0·35	0·35
	Adrensine triphosphate	3·53	2·10	0·48	0·17	0·08
	Total	8·35	7·90	7·99	8·03	8·21
Red lateral muscle	Hypoxanthine	0·35	0·17	0·52	0·85	1·25
	Inosine		0·53	1·90	3·11	3·42
	Adrenosine monophosphate	0·12	0·20	0·17	0·12	0·15
	Inosinic acid	1·07	3·21	1·86	1·34	0·62
	Adrenosine diphosphate	0·93	0·44	0·14	0·30	0·30
	Adenosine triphosphate	3·39	1·30	0·00	0·53	0·51
	Total	5·86	5·85	5·85	6·25	6·26

Values are expressed as μ moles/gm. muscle wet-weight

The second is dependent upon the degree of splitting of inosinic acid. In red lateral muscle there is a rapid splitting of inosinic acid as time goes by while in dorsal the same phenomena as in carp muscle (accumulation of inosinic acid) are observed, and, as a result, the amounts of inosine and hypoxanthine in the former increase rapidly.

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PLANT PHYSIOOGY

Plant Growth Activities of 5- and 8-Halogeno-dihydro- and -tetrahydro-1-naphthoic Acids

IT is known that dihydro- and tetrahydro-1-naphthoic acids have high plant growth activities[1]. With the view of testing the effect of substitutions on the activities of these acids, we synthesized chloro- and bromo-derivatives in the 5- and 8-positions of 1,4-dihydro, 3,4-dihydro- and 1,2,3,4-tetrahydro-1-naphthoic acids and tested their activities in the pea straight-growth test[2].

Table 1. Activities of Hydro-1-naphthoic Acids and 5- and 8-Halogen Substituted Hydro-1-naphthoic Acids in Pea Straight Growth Test

Final length as percentage of control

Compound (-1-napthoic acid)	Concentration (mgm./l)†								
	0	0·1	1	10	30	50	100	200	300
1,4-Dihydro-	100	103	135	129	133	129	128	—	115
3,4-Dihydro-	100	100	99*	105	127	130	123	—	115
1,2,3,4-Tetrahydro-	100	99*	120	140	135	136	134	—	113
5-Chloro-1,4-dihydro-	100	—	106	131	—	123	114	108	—
5-Chloro-3,4-dihydro-	100	—	98*	106	114	112	105	—	102*
5-Chloro-1,2,3,4-tetrahydro-	100	100	100	112	126	124	106	—	101*
5-Bromo-1,4-dihydro-	100	—	104	133	135	125	115	—	103
5-Bromo-3,4-dihydro-	100	—	—	103	103	107	104	—	101*
5-Bromo-1,2,3,4-tetrahydro-	100	—	100	108	128	128	112	—	101*
8-Chloro-1,4-dihydro-	100	—	100	134	131	131	126	—	113
8-Chloro-3,4-dihydro-	100	100	116	136	131	127	120	—	108
8-Chloro-1,2,3,4-tetrahydro-	100	100	124	134	127	128	125	—	110
8-Bromo-1,4-dihydro-	100	—	100	132	136	134	128	—	118
8-Bromo-3,4-dihydro-	100	—	110	140	136	128	118	—	110
8-Bromo-1,2,3,4-tetrahydro-	100	—	109	134	130	127	114	—	104

* Difference from control is not significant at 1 per cent level.
† The discussion scarcely affected if concentrations are expressed as moles/l.

5-Halogeno-1,2,3,4-tetrahydro- and -1,4-dihydro-1-naphthoic acids were prepared by the Sandmeyer reaction from the corresponding 5-amino-hydro-acids, derived from 5-amino-1-naphthoic acid by sodium amalgam reduction. 5-Halogeno-3,4-dihydro-1-naphthoic acid was obtained by boiling the 1,4-dihydro-acid with alkali. 8-Halogeno-hydro-1-naphthoic acids were prepared in a similar way from naphthostyril[3].

Activities in the pea straight-growth test of these acids, as well as of their parent acids, are presented in Table 1. The results show that the chlorine and the bromine substitutions are similar in their effect on the growth activity. In the 1,4-dihydro-acid series, both the 5- and 8-substitutions make the threshold concentration higher. In the 3,4-dihydro-acid series, the 5-substituted acids are less active and the 8-substituted acids are more active than the parent acid. In the 1,2,3,4-tetrahydro-acid series, the 5-substitution depress the activity considerably, while the 8-substitution do only slightly.

Veldstra has proposed as the structural requirement for the plant growth substance, the presence of a lipophilic ring system (*L*) and a hydrophilic group (*H*) in a very definite spatial relation with each other, and an appropriate *HL*-balance[4]. We will discuss our results from this point of view.

Of the non-halogenated hydro-1-naphthoic acids, 1,4-dihydro- and 1,2,3,4-tetrahydro-acids are similar in their activities, while 3,4-dihydro-acid is less active than the two. As reported in the previous paper, the surface activities (half suppression value) of the three acids do not differ appreciably from one another, the order being 1,4-dihydro-acid 3,4-dihydro-acid 1,2,3,4-tetrahydro-acid[5]. Therefore, the marked difference in the growth activity may be attributable mainly to differences in the spatial position of carboxyl group with respect to the ring system. The carboxyl group may be nearly coplanar with the ring system in the 3,4-dihydro-acid because of the conjugated structure, while in the 1,4-dihydro- and 1,2,3,4-tetrahydro-acids the group can take, through interconversion of the alicyclic ring, two positions, in one of which the carboxyl group rises up considerably from the ring plane (quasi-axial conformation)[6].

By the 5-substitution, the activities of the three acids are depressed in parallel with each other. This may be explained by a shift in the *HL*-balance, because that kind of substitution may have no significant steric effect on the carboxyl group.

On the other hand, all the 8-substituted acids are nearly equally active. Due to the steric effect of the substituent in 8-position, the carboxyl group may exist predominantly in quasi-axial position in 1,4-dihydro- and 1,2,3,4-tetrahydro-acids and it may be forced to twist and rise up more or less from the ring plane in 3,4-dihydro-acid. Such a steric effect may perhaps overcome the suppressive effect of a shift in the *HL*-balance in 3,4-dihydro-acid and nearly compensate for it in 1,4-dihydro- and 1,2,3,4-tetrahydro-acids.

Thus, the present results are consistent with Veldstra's hypothesis. However, syntheses and physiological tests of 6- and 7-substituted acids are desirable. It is also hoped to determine more precise molecular structures and surface chemical properties of the acids in question. The chemical reactivities of definitie positions of the aromatic ring should also be considered in connexion with Muir and Hansch's hypothesis[7].

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Effects of Substituted Phenoxy-Compounds and Other Translocated Herbicides on the Rhizome of Bracken

It is now widely recognized that the food reserves in the underground stem of bracken, *Pteridium aquilinum*, are often greater than was formerly realized, and that defoliation, even if prolonged, is frequently insufficient to kill the growth-centres[1–3]. Since the underground stem is the organ of growth and extension as well as of perennation, control of the plant's development should be sought by direct rather than indirect control of such an active and massive organ.

Table 1. STATE IN SEPTEMBER OF UPPER-LEVEL BRANCHES OF BRACKEN RHIZOME COMPARED AFTER CUTTING AND AFTER TREATMENT WITH 4-CHLORO-PHENOXYACETIC ACID (4-CPA)

Treatment	Stage of Bracken Growth	Branch apices associated with: Current year fronds — Rhizome tips/20		Associated Frond-buds on apex			Fronds older than 1 year — Rhizome tips/10		Associated Frond-buds on apex		
		Healthy	Dead or dying	Healthy	Dead or dying	Not formed	Healthy	Dead or dying	Healthy	Dead or dying	Not formed
Untreated control (mean from 9 plots)		20	—	17	—	4	10	—	9	—	1
Cut in late June (mean of 5 plots)	Not fully mature	20	—	14	—	6	10	—	7	—	3
Chemical (lb./acre) 4-CPA—5 lb. (mean of 60 plots)	Not fully mature	2	18	2	12	6	6	4	4	3	3
	Mature	1	19	1	13	6	3	7	2	5	3
4-CPA—7½ lb. (mean of 40 plots)	Not fully mature	2	18	1	10	9	6	4	4	3	3
	Mature	1	19	1	15	4	3	7	2	5	3
4-CPA—10 lb. (mean of 38 plots)	Not fully mature	1	19	1	11	8	4	6	4	4	3
	Mature	—	20	—	13	7	1	9	1		

Chemical treatment of bracken-infected land as a more direct mode of control has been attempted for more than 20 years[4–6], but there are few records of the effects produced on the underground stem. Early tests were carried out with contact herbicides such as sulphuric acid and sodium chlorate; and both were, in the main, reported as unequal and unsatisfactory. More recent trials, analysed by direct observations on the rhizome, after treatment with contact herbicides such as sodium chlorate, boron compounds and mixtures of the two, confirm the earlier reports since they show that damage occurs to the rhizome only when the toxic compounds are in direct contact in massive doses[7,8].

With increased knowledge of compounds translocated through plant systems that may act as promoters of irregular growth of certain organs, a new method of control is suggested; and in 1956, it was reported[7] that rhizomes of bracken when young and active showed formative effects after frondular spraying with the phenoxy-acetic compounds 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chlorophenoxyacetic acid (4-CPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their butyric homologues. Thus the possibility of translocation through a young storage organ was established. In these tests, 4-CPA and 2,4,5-T appeared to be particularly effective on the rhizome of the plant.

During the period 1955–58, an extensive series of trials has been carried out on test-plants of various ages and on mature stands of field bracken. Analyses of the rhizome have been made by direct observation[8] and it is now clear that (1) many compounds, such as ammonium sulphamate, are vigorous defoliants but there is little translocation through the mature plant system and no direct effect on the mature underground stem from foliar spraying. (2) Gibberellic acid, aminotriazole, sodium 2,2-dichloropropionate (dalapon) and MCPA, 2,4-D, 4-CPA, 2,4,5-T, 2-(4-chloro-2-methyl phenoxy) propionic acid (CMPP), 2-chloro-4-fluorophenoxyacetic acid and 2,4-dichloro-5-fluorophenoxyacetic acid are translocated to the rhizome when applied as foliar sprays, and some are able to induce formative effects. Thus, (*a*) gibberellic acid has a stimulatory effect though no marked changes in the growth pattern have yet been observed; (*b*) aminotriazole retards growth and induces a chlorotic condition in the fronds in the first and second years after treatment though no systemic effects on the rhizome or rhizome apices have been detected; (*c*) dalapon has a retarding effect on frond-bud extension[10,11] but causes no formative disturbances to the rhizome apex[8] though internally the amount of sclerotic tissue increases markedly; (*d*) the phenoxy-compounds cause marked epinasty in young fronds and deformation of the apical meristems of both rhizome and frond-buds, with splitting of the outer tissues of these organs: the latter effect may lead to break-down and rotting of internal tissues. (3) Translocation of the phenoxy-compounds is marked along axes apical to a treated frond: it is more restricted into side branches not directly connected with an expanded frond.

Following the early promise of 4-CPA, more extensive tests have been carried out, with several formulations, on 2,-3-year-old plants, on transplanted fragments of rhizome and on stands of field bracken, in an attempt to extend our appreciation of the way in which this growth-regulating chemical acts on the plant. One aspect of interest is the difference induced on the growing-centres of the plant by mechanical treatment and by spraying with such a translocated compound as 4-CPA; and the results at the end of the first year of such a test are shown in Table 1.

This test was made on plots of bracken (30 × 4 yd. with a 3-yd. boundary between plots to prevent interference), and the results (*a*) confirm earlier observations that 4-CPA is able to induce a direct attack on the rhizome of field bracken by its penetration to the branch apices, and (*b*) suggest that later spraying when the fronds are fully mature (mid-end July in Scotland) is more effective than treatment of young, just unfolded leaves (late June in Scotland). This may be due to reduced translocation because of the severe scorch produced on young fronds, and which older fronds are able to withstand.

These tests will shortly be reported more fully. Though many questions are still outstanding, these preliminary trials suggest that certain compounds can act as translocated agents of control, and such compounds therefore offer a new approach to an important agricultural problem which has been of long standing.

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HÆMATOLOGY

Two Hæmoglobins in Chum Salmon

Occurrence of multiple hæmoglobins has been reported recently in fish as well as in other vertebrates. In particular, Salmonidæ was found electrophoretically to contain fairly close amounts of two or more components[1,2]. Some physico-chemical properties of the two components of blood hæmoglobin in the salmon *Oncorhynchus keta*, which were prepared from hæmolyzate by starch zone electrophoresis, have already been reported[3]. The fast-migrating component in the physiological *p*H region (designated component *F*) was more labile to heat denaturation, less soluble in concentrated salt solution, and behaved less anionically in electrophoresis than another component (designated as component *S* in view of its slow migration). Their behaviour in column chromatographic and absorption spectra in the Soret region were also more or less different from each other.

The present communication deals with crystallization and several properties of the two components. Hæmolyzate from chum salmon blood saturated with carbon monoxide, was 0·35 saturated with ammonium sulphate at neutral *p*H and suctioned. The filtrate was 0·6 saturated with the salt and the resulting precipitates were collected, dissolved in distilled water, and the solution was made slightly turbid by adding ammonium sulphate at *p*H 6·8–6·9, and left standing overnight in a cold place, then the crystals of component *F* appeared in trapezoid or square-shaped form. From the filtrate of the solution, component *S* was crystallized by the similar procedure, but at slightly lower *p*H (about 6·3) and after slightly longer period of standing (usually 2–3 days). This component was obtained in clusters of needle-shaped crystals (Fig.1).

Both preparations, after recrystallized twice, were found to be practically homogeneous in moving boundary electrophoresis and then analyzed for sedimentation and diffusion constants in a phosphate buffer 0·1 *M*, *p*H 7·0. Molecular weight and frictional ratio were also calculated using these constants and assuming partial specific volume for this protein of 0·750. As seen in Table 1, there are appreciable differences in these values between the two components. The sedimentation and diffusion constants and the frictional ratio of component *F* are in a good agreement with those reported for vertebrate hæmoglobins[4], whereas those of component *S* are not. It is, however, a question whether the same relation holds for molecular weight or not, considering a fairly large variation in the values calculated by such means for mammalian hæmoglobins (63,000–76,000[5]). The axial ratio of component *S* is more than double that of component *F*, namely *S*10·2,*F*4·6 (as prolate) or *S*12·8,*F*4·8 (as oblate). Thus it was proved that both components are different in molecular shape as expected from the results obtained previously[3].

Table 1. Some physical properties of two components *F* and *S*

	Component *F*	Component *S*
Sedimentation constant, $s_{20,w}$ (*S*)	4·16	3·64
Diffusion constant, $D_{20,w}$ (cm.²/sec.)	$6·6\times10^{-7}$	$4·9\times10^{-7}$
Molecular weight, $M_{s,D}$	61,000	72,000
Frictional ratio, f/f_0	1·23	1·56

Another difference was observed also in the function as the respiratory pigment between them. Oxygen dissociation curve was measured in phosphate buffers of various *p*H's (7·75–6·6) for both preparations isolated from hæmolyzate by starch block zone-electrophoresis. The effect of *p*H on the oxygen dissociation curve was observed for component *F*; a decrease in *p*H of the solution from 7·75–6·6 resulted in such drastic lowering in oxygen affinity that most of the hæmoglobin remained reduced even in the atmospheric pressure. On the contrary, the oxygen affinity of component *S* was scarcely affected by such a change in *p*H. Details of this work will be published elsewhere.

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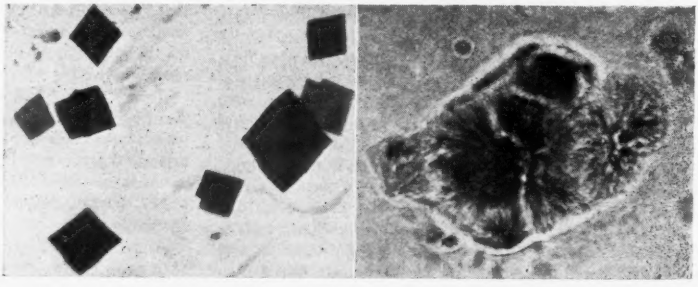

Fig. 1. Crystals of component *F* (left, ×75) and *S* (right, ×410) of salmon hæmoglobin

Neutralization of Bridge Blood Anticoagulant

In order to correct the deficient plasma thromboplastin generation in hæmophilia, extraordinary amounts of antihæmophilic globulin are required[1]. This has been attributed to the inhibitory action of Bridge anticoagulant[2]. The fact that the latter is characteristically absent in serum[3] prompted further investigations on the changes in the concentration of this inhibitor during the process of coagulation. The results (Table 1) revealed that full activity was maintained until the clotting of fibrinogen, following which event the anticoagulant rapidly became inert. Theoretically this meant that the removal of Bridge anticoagulant may be accomplished by one of three processes; adsorption on fibrin, destruction by thrombin or inactivation by a compound formed by thrombin-fibrinogen interaction.

Table 1. The effect of coagulation on the concentration of Bridge anticoagulant

Time of testing*	Clotting time of substrate (sec.)†
Before clotting	23½–25
1 minute after clotting	12
15 minutes after clotting	11

* Venous hæmophilic blood was rapidly drawn and aliquots of 1·8 ml. incubated at 37°C. At 2-min. intervals, the clotting process was stopped in one of the specimens by the addition of 0·2 ml. 3·8 per cent sodium citrate. The activity of Bridge anticoagulant in each specimen was then estimated.

† Plasma (or serum) to be tested was treated with aluminium hydroxide and then incubated undiluted with equal volumes of factor V solution (5 mgm./ml. imidazole buffer pH 7·3), antihæmophilic globulin solution (0·3 mgm./ml.), platelet suspension, imidazole buffer *p*H 7·3, diluted (1:10) normal serum and 0·05 *M* calcium chloride. The activity of plasma thromboplastin formed after 6 min. was estimated by adding 0·1 ml. of the mixture together with 0·1 ml. 0·025 *M* calcium chloride to 0·1 ml. substrate (normal citrated plasma), recording the clotting time. Normal reagents gave 10 sec.

Further experiments were, therefore, carried out in order to test the above possibilities. Defibrination by the addition of small amounts of thrombin completely abolished the anticoagulant activity (Table 2). On the other hand, neither removal of fibrinogen by heat at 56°C. for 3 min., nor the addition of thrombin to heat-defibrinated plasma affected the inhibitory action. This denoted that the agent responsible for the neutralization of Bridge anticoagulant is derived from the action of thrombin on fibrinogen.

Table 2. The effect of defibrination on Bridge anticoagulant activity

Method of defibrination	Substrate clotting time (sec.)*
Thrombin	11
Heat (56°C.)	24
Control (no defibrination)	25

* For details see Table 1.

Methods have now been developed for the preparation of this Bridge anticoagulant neutralizing agent, free of antihæmophilic globulin, from fresh blood as well as from expired bank-blood. *In vitro* experiments showed that, by removing the action of Bridge anticoagulant, this material improves the action of antihæmophilic globulin; a much smaller amount (20 per cent) of the latter factor was required for the production of normal levels of plasma thromboplastin from undiluted hæmophilic plasma (unpublished work).

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PATHOLOGY

Hereditary Absence of Spleen in the Mouse

The 'luxoid' mutants of the house-mouse form a small and well-defined group with striking limb-defects. In luxate[1] (*lx*) and luxoid[2] (*lu*) homozygotes hind-legs are often twisted, due to reduction or loss of the tibia and distortion of the fibula with associated muscular abnormalities. The femur may also be shortened and fragmented and even the pubic element of the pelvic girdle may be affected in luxates. The hind-feet may show pre-axial poly- or oligo-dactylism, the fore-feet may also be polydactylous in *lu lu* mice. Heterozygotes may appear normal or may have pre-axial triphalangy or polydactyly of the hind-feet. There are various other skeletal effects (on the number of presacral vertebræ, etc.) while the urogenital system is also involved in luxate homozygotes, with frequent hydronephrosis and hydroureter.

Among other members of this group which have recently been investigated is one in which the skeletal and other defects are decidedly more severe. T. C. Carter has shown that this 'dominant hemimelia' (*Dh*) is close to leaden (*ln*) in linkage group XIII[3].

Skeletally, *Dh* heterozygotes may be affected as severely as luxate homozygotes[4] and in much the same way. Thus the hind-limbs may show the following abnormalities: (1) pre-axial triphalangy, poly-, syn- or oligo-dactyly; (2) tibial hemimelia or absence with associated fibular distortion, so that the leg is twisted and useless; (3) reduction and sometimes fragmentation of the femur; (4) reduction or even loss of the pubic element of the pelvic girdle, though this only rarely occurs. The number of sternebræ and of presacral vertebræ also tend to be reduced. Limb abnormalities are generally more severe in *Dh* homozygotes, with loss of up to three digits from the pre-axial side of the hind-foot, while the hind-leg is always greatly twisted and reduced in size. The fore-limb is unaffected, however.

Visceral examination of *Dh* heterozygotes and homozygotes has revealed some interesting abnormalities. All entirely lack the spleen. This can be seen without dissection in newly born or day-old mice, when the viscera are still visible through the body-wall and the bright red colour of the spleen contrasts with the dull red of the adjacent kidney. Heterozygotes also tend to have a smaller stomach and a shorter alimentary canal than normal; moreover, they show a marked flattening of the antero-ventral part of the left kidney. Hydronephrosis and hydroureter may occur, as in *lx lx* mice, but are not common. Lymphatic tissues other than the spleen, such as the intestinal Peyer's patches and the thymus, seem unaffected.

Dominant hemimelia is lethal when homozygous, death usually occurring within four days of birth. Homozygotes are obtained by intercrossing *Dh ln*/+ + mice; since these two loci are only about 5 units apart, 90 per cent of *Dh Dh* offspring will also be leaden. This allows a useful check on the genotype of presumed homozygotes, since leaden homozygotes can be identified at birth by microscopical examination of the skin, when very marked clumping of pigment granules can be seen in the hair follicles.

Besides the spleen loss and limb defects already mentioned, the nine homozygotes examined so far have had serious urogenital and alimentary defects. These include severe hydronephrosis and hydroureter, the swollen ureters often joining together and ending

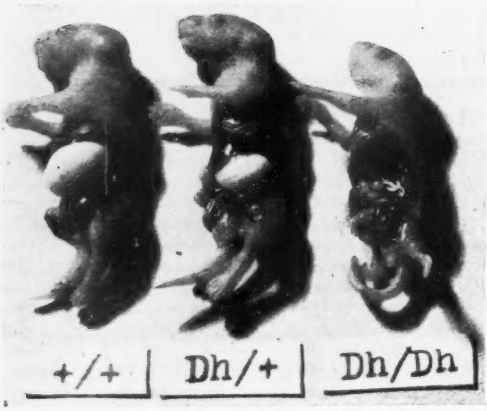

Fig. 1. Day-old mice opened to show: (1) lack of spleen and smaller stomach than normal in *Dh*/+; (2) lack of spleen, minute stomach, hydronephrosis with hydroureter, small and twisted hind-limbs in *Dh*/*Dh* mouse

blindly, with no trace of a bladder and without a fully formed urethra. Testes and vasa deferentia are present, but sometimes there are no external genitalia, while the anus and external opening of the urethra may be closed. The size of the stomach is always greatly reduced, while the intestine is about half its normal length and usually ends blindly in the region of the colon. Thus the lethality of homozygotes is not surprising. Fig. 1 compares viscera in homozygote, heterozygote and homozygous normal.

Dh/+ mice were crossed with homozygous normals and the offspring classified as soon as possible after birth for spleen loss and pre-axial limb defects. 37 had both, 40 had neither, none had one type of defect without the other; this is a close approximation to the expected 1 : 1 ratio of affected and normal. Of 46 offspring from intercrosses of heterozygotes, classified as above, 13 were normal, 5 had severe limb defects and no spleen (*Dh*/*Dh*), 27 had milder limb defects and no spleen (*Dh*/+) while one lacked a spleen but had normal limbs. This last mouse was presumably a heterozygote in which the limb defects had failed to manifest themselves; a few such animals are to be expected, as visible limb defects in heterozygotes are sometimes very small: perhaps just a slight elongation or thickening of one hallux. The observation of only five presumed *Dh* homozygotes when expectation is 11·5 suggests that some die and are eaten very soon after birth, before they can be recorded. This may be associated with severe umbilical hernia, since an example of this has been found in a *Dh*/*Dh* fœtus, with protrusion of much of the liver and some gut.

The neonatal death-rate of *Dh* heterozygotes seems to be higher than normal; but those surviving into adult life show little or no reduction in viability or fertility. Even those with twisted and useless hind-legs seem able to mate satisfactorily. The spleenlessness of these heterozygotes should make them useful subjects for radiobiological and physiological research.

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Antibody Status of Mice and Response of their Litters to Parotid Tumour Virus (Polyoma Virus)

In earlier work there was observed a tendency for pleomorphic tumours of the parotid gland to appear only in certain litters of *C3H* mice that had received inoculations of preparations from leukæmia tissues[1]. This 'litter effect' was also observed in tests with a more active preparation of tumour-inducing material propagated in a continuously passaged tissue culture line of a murine leukæmia, *P*-388D_1[2].

When it was found that the breeding colony had a moderate incidence of spontaneous infection with parotid tumour virus[3,4], it appeared desirable to determine if the 'litter effect' was a reflexion of the immune status of the mothers. Other workers have shown that suckling mice are protected from tumours by admixture of the virus inoculum with rabbit immune serum[5] and that offspring of immunized hamsters are relatively refractory to infection.[6]

Seventeen *C3Hf*/$_{Lw}$ females were mated at 2 months of age to *AKR*/$_{Lw}$ males. At birth of the first litter these mothers were bled and their sera tested for hæmagglutination-inhibiting antibodies by methods previously described[4]. Sera were again tested at weaning of the first litter and at birth and weaning of subsequent litters.

All litters were inoculated sucutaneously within 48 hr. of birth with 0·05 ml. of undiluted tissue culture fluid of the tenth passage of parotid tumour virus, passaged in *P*-388D_1 leukæmia tissue cultures. This represents an inoculum of $10^{4\cdot9}$ adult mouse ID_{50} per animal, as determined in the mouse antibody production test[7]. The origin of this strain of virus has been described elsewhere[2]; hæmagglutination by the strain is specifically inhibited by mouse antiserum to the *LID*-1 strain[7], which was used for all serological testing.

Hæmagglutination-inhibiting antibody was detected in 4 of these 17 *C3H* mothers at the birth of their first litters. There was no history of cage contact with inoculated mice. Twenty-two (*C3H* × *AKR*)F_1 mice comprising the 4 litters from hæmagglutination-inhibiting mothers were inoculated with parotid tumour virus and all have remained free from tumours for 7 months. Also, 27 mice born as second litters of these 4 mothers and inoculated at birth with parotid tumour virus have remained free of tumours for 6 months.

In contrast, 11 of the 13 antibody-negative *C3H* mothers bore first litters and 64 per cent of the 77 F_1 mice have developed parotid gland tumours and in certain instances other grossly visible tumours within a 7-month observation period following inoculation with parotid tumour virus. Tumourous mice were found within all 11 litters. Nine of these mothers bore second litters, which were also inoculated with the standard virus preparation. Six of the 9 mothers had become serologically positive by the time of birth of their second litters; two of the 6 serologically positive mothers had third litters. Seventy-seven second-and-third-litter mice representing these 8 litters were inoculated at birth with the same parotid tumour virus preparation and have remained free from tumours for 5–6 months. Three mothers failed to convert serologically following suckling of infected first litters, and their second litters remained susceptible to virus (Table 1). Previous studies have shown that inoculated sucklings excrete large quantities of virus[3,4] and that the mothers have correspondingly high rates of spontaneous infection[8].

Table 1. ANTIBODY-STATUS OF *C3H* MOTHERS AND RESPONSE OF F_1 LITTERS TO PAROTID TUMOR VIRUS

No. of mothers	HI antibody status	1st Litters: No.	No. mice	No. with parotid gland tumours	Latent period (months)	2nd and subsequent litters: HI antibody status at 2nd & subs. litters	No.	No. mice	No. with parotid gland tumours	Latent period (months)
17	13 negative	11	77	49	1½ to 5	3 negative	3	24	12	1½ to 4
						8 positive*	8	77	0	—
	4 positive	4	22	0	—	4 positive	4	27	0	—

* 6 serologically positive females bore 2nd litters and 2 of these 6 had 3rd litters.

Table 2. TUMOUR—AND ANTIBODY-STATUS OF $(C3H \times AKR)F_1$ MOTHERS AND RESPONSE OF F_2 LITTERS TO PAROTID TUMOUR VIRUS

No. of mothers	Tumour status	HI antibody status	No. litters	No. of offspring	No. with thymic tumours	Latent period (months)
15	Parotid tumour	Positive	15	134	0	—
14	None	Negative	14	118	111	1 to 5

Four litters totalling 23 F_1 mice, which failed to develop tumours and which were obtained from serologically positive mothers, were tested for hæmagglutination-inhibiting antibody at 10 weeks of age, 6 weeks after weaning. All members of 3 of these litters were negative (<100) whereas 3 of the 5 offspring of the fourth litter were positive (>200) at this time. In contrast, offspring from negative mothers invariably developed hæmagglutination-inhibiting antibodies following inoculation in the neonatal period with parotid tumour virus.

In a second experiment, using $(C3H \times AKR)F_1$ mothers, a comparison was made of the response of litters born to tumour-bearing, inoculated mothers and non-tumourous, uninoculated mothers. First litters were obtained from fifteen two and one-half to four-month old F_1 mothers bearing parotid tumours, and from fourteen normal F_1 mothers four months of age. The tumourous mice were all antibody positive (>200), and the normal F_1 mothers were all negative (<100). The litters received passage 1 of $P\text{-}388D_1$ propagated parotid tumour virus within 48 hr. of birth. This material was used after 6 months storage at −70° C and 10^{-2} dilution. The results are shown in Table 2. F_2 litters from serologically positive F_1 mothers have remained free of tumours for eight and one-half months, whereas 111 of 118 F_2 offspring (94 per cent) from serologically negative mice presented epithelial tumours of the thymus within a 5-month period, the earliest appearing at less than 1 month. Tumour-bearing mice were found within all 14 litters. Small salivary gland tumours were observed grossly in only 3 of these mice, although microscopic examination disclosed early salivary gland tumours in most animals in which they had not been detectable grossly. It is interesting that tumours of ectopic thymuses were observed in several mice. The striking thymotropic character of this preparation, given at 1:100 dilution, was observed before[2].

Foster-nursings of litters born of antibody-positive *C3H* mothers by antibody-negative mothers and vice versa have been accomplished in ten instances. Parotid tumour virus of the tenth passage material was inoculated 6 hr. after the transferred young had suckled their foster mothers. In 6 such reciprocal transfers the young have now reached the age of 5–7 months and all remain free of tumours, indicating that the transmission of antibodies from the mother to her young occurs in the prenatal period as well as through the mother's milk. Tests for hæmagglutination-inhibiting antibodies in fœtal serum, milk, and serum of foster-nursed sucklings likewise demonstrated efficient transfer of the antibody both transplacentally and in milk[8]. Some previous evidence has indicated that the passive transmission of antibodies in the mouse is similar to that in the rat; transmission occurs from mother to fœtus and also during a period after birth[9].

These results show a striking correlation between hæmagglutination-inhibiting antibody status of the mother and susceptibility of the litter to tumour induction by virulent parotid tumour virus. Natural infection with parotid tumour virus, apparently through room or cage contact and from suckling of an inoculated litter, as well as artificial contact through inoculation of mothers at birth, has resulted in the levels of hæmagglutination-inhibiting antibody in the mother capable of protecting entire litters from infection with parotid tumour virus. Preliminary results of reciprocal foster-nursings indicate passive transfer of immunity to the fœtus and through the mother's milk.

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BIOLOGY

Relict Fauna of Ennerdale Water; a Problem of Distribution

THE presence in Ennerdale Water, Cumberland, of the two crustaceans, *Mysis relicta* and *Limnocalanus macrurus*, characteristic of 'relict' lakes which formed part of arctic or sub-arctic seas during the closing stages of the last glacial period, has given rise to speculations about the possibility of a former connexion between this lake and the sea[1,2]. Since Ennerdale Water, unlike these relict lakes, is 368 ft.

Table 1. Correlation of Ennerdale cores with late-glacial and early post-glacial events, based on Godwin and Eastwood (ref. 1). Radiocarbon dates from Godwin *et al.* (ref. 7)

	Baltic Stages	Pollen Zones	Climatic Period	North-west England	Ennerdale		Radio-carbon Dating
							B.C.
POST-GLACIAL	Littorina Sea	VII	Atlantic	Clay and silt bands in lakes	Diatom Gyttja	Expansion of Alnus	5,200
	Ancylus Lake	VI	Boreal			Corylus maximum	6,875
		V				High Salix	7,600
	Yoldia Sea	IV	Pre-Boreal				8,300
LATE-GLACIAL	Ra moraines	III	Younger Dryas	Corrie Glaciation		Laminated Clay	8,800
		II	Alleröd	Birch woodland widespread		Clayey silt with freshwater diatoms Late-glacial flora	10,000
	Baltic End moraine Daniglacial	I	Older Dryas	Scottish Readvance (Upper Boulder Clay)		Laminated Clay	
FULL-GLACIAL				New Drift Glaciation (Lower Boulder Clay)		Glacier-Lake Ennerdale	

above sea-level—far beyond the amplitude of any glacial or post-glacial changes in the relative level of land and sea—the suggestion was that sea water was possibly pushed up the valley by ice. Recent investigations of cores from the bottom deposits of Ennerdale Water have provided proof that during the closing stages of the last glacial episode, no sea water was present in this lake.

Geologically, Ennerdale Water is unique among the lakes of the English Lake District in showing evidence of the former occupation of part of its present basin by a Glacier-Lake Ennerdale, which formed during the retreat stages of the New Drift Glaciation, when melt-water from the Lake District ice-cap was dammed up against the Irish Sea ice. Gravels in the Ennerdale valley are now recognized as deposits of Glacier-Lake Ennerdale, and not as marine gravels as was at one time supposed. Glacier-Lake Ennerdale drained and disappeared at the close of the New Drift Glaciation, and never again formed.

At some time considerably after the New Drift Glaciation, a re-advance of the shrunken Scottish ice-sheet brought its margin once again on to the coastal strip of north-west Cumberland. Eastwood[3] suggests that "during the advance [the Scottish re-advance] some sea water was probably trapped and forced inland, perhaps as far as Ennerdale". Tattersall[2] in his discussion of this suggestion, confuses the Scottish re-advance with a minor re-advance of the Irish Sea ice during the preceding (New Drift) episode, which had temporarily raised the level of Glacier-Lake Ennerdale. In fact, of course, Glacier-Lake Ennerdale had disappeared long before the Scottish re-advance, so no weight can be attached to Tattersall's speculations as to whether sea-water may have been introduced into the present lake via Glacier-Lake Ennerdale.

During the Scottish re-advance, it seems certain that the severity of the climate maintained valley glaciers in the Lake District. The laminated clay at the base of the Windermere deposits has been assigned to this period, which is equivalent to Zone 1 of the Late-glacial period. In a core from the western, shallow end of Ennerdale Water, the stratigraphy and pollen analysis show that the sequence, from Zone 1 right through to the post-glacial deposits, is present and conformable and corresponds closely to the stratigraphical succession described from the north basin of Windermere[4].

In both lakes, Zone 1 is represented by a thick deposit of glacial clay with conspicuous laminations and no organic remains except occasional eroded shells of the freshwater diatom *Melosira arenaria*; Zone II by a clayey silt containing plant remains and abundant freshwater diatoms, and Zone III (the period of the corrie glaciation of the Lake District) by a clay with some laminations and containing some freshwater diatoms. The diatom species are strikingly similar in both lakes, and in both, the first plants to appear in the deposits as the severe conditions of Zone I gave way to the milder conditions of Zone II are the diatom *Melosira arenaria* and the moss *Fontinalis squamosa*. (These two species are now sometimes found in association in calcareous springs, the diatom epiphytic on the moss[5].) These species, and all the other species of diatoms and mosses identified from Zone II in Ennerdale, are undoubtedly entirely freshwater or the inhabitants of mountain rocks. Pollen is very sparse in both Zone I and Zone III clays, but in the silt of Zone II in Ennerdale were found the pollen grains of a typically late-glacial assemblage of plants—*Betula* (not *B. nana*), *Myriophyllum alterniflorum*, *Filipendula ulmaria*, *Thalictrum*, *Artemisia*, *Juniperus*, *Helianthemum*, and the pollen of the Gramineæ and Cyperaceæ. This confirms the identification of these layers in Ennerdale as representing the late-glacial period.

The laminated clays of Zones I and III in Ennerdale are, like those of corresponding age in Windermere, true diatactic varves, each having a lower portion of coarse and an upper portion of fine material, with a very sharp boundary between the fine material and the overlying coarse layer. Scandinavian geologists have shown that such diatactic varves can only form in fresh water, since a salinity greater than 1/50 normal sea water causes flocculation, and precipitation of a single unsorted layer. The presence of a considerable thickness of diatactic varves, dating from the earliest phase of the late-glacial, in Ennerdale Water, therefore effectively disproves any theory that sea-water was trapped in the lake during the Scottish re-advance. Furthermore, since these varved clays contain no remains of planktonic organisms, and their carbon content is practically nil, it is not possible to suppose that *Limnocalanus* and *Mysis* survived through the period from an earlier introduction.

It is, of course, during the early stages of the post-glacial period that the isolation of *Mysis* and *Limno-*

calanus in the Baltic relict lakes is supposed to have occurred. It is thought that *Mysis oculata* and *Limnocalanus grimaldii* in the Yoldia Arctic sea became modified during the succeeding period of the freshwater Ancylus lake into forms resembling *Mysis relicta* and *Limnocalanus macrurus*, which became isolated in the smaller freshwater lakes which were cut off from the arms of the Ancylus lake by subsequent changes in relative level of land and sea. In this early post-glacial period, covering Zones IV, V and VI of the pollen zonation, the stratigraphy and pollen diagram of this core from the western end of Ennerdale correspond closely with the succession in Windermere, and show that the sequence of deposits is complete and conformable. The curves for pollen of the forest trees show the normal course for these zones in north-west England, and the horizon of the Boreal/Atlantic transition, Zone VI/VII, is clearly marked at 450 cm. below the mud surface. Throughout this period of Zones IV, V and VI, abundant remains of freshwater diatoms make up a significant proportion of the deposits, and prove that not even brackish water was present. The diatoms belong to species identified from deposits of similar age in Windermere and Kentmere[4,6]; many of them are epiphytes on freshwater plants. It can be taken as proved beyond doubt that at the time when, in the Baltic, changes in land/sea-level and alternations of salt and fresh water were leading to the isolation of *Mysis relicta* and *Limnocalanus macrurus* in relict lakes, Ennerdale Water remained a deep freshwater lake of approximately its present shape. So the reason for the presence here of these two crustaceans must be different from that which has been put forward for the relict lakes.

Finally, a survey of the bottom deposits of the deep, upper, eastern basin of Ennerdale Water has disposed of any suggestion that sea-water may have been trapped here. Slumping of clay and organic muds down the steep sides of this basin has destroyed the conformable succession at the boundary between late- and post-glacial periods, but below the slumped and mixed-up layers of Zones II, III and IV, lie more or less horizontal laminated clays, which contain no organic remains and prove that no sea-water was in the basin when they were formed. The slumped deposits of Zones II, III and IV, and the overlying organic muds of Zones V, VI and VII, contain abundant freshwater diatoms of the same species as in Windermere, and show no evidence of a different history from that lake.

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Life-Cycle of *Cyathostoma lari*, Blanchard 1849 (Nematoda, Strongyloidea)

During an investigation into helminth parasitism in wild birds in northern England, specimens of an unusual nematode, *Cyathostoma lari* E. Blanchard 1849, were recovered from a variety of host species. *C. lari*, a parasite of the nasal and orbital sinuses of gulls[1,2], has been recorded only at infrequent intervals in various parts of Europe and was not known to occur in Britain until Burt and Eadie[3] recovered it from *Larus ridibundus*, *L. cinereus*, *Corvus frugilegus*, *C. monedula*, *C. corone*, and *Tringa totanus* in Scotland (St. Andrews). I have also found it in the first four hosts listed above and additionally in *Sturnus vulgaris* and *Ardea cinerea*.

Since little is known of the life-cycle of any species of *Cyathostoma* it was decided to investigate its mode of transmission. However, since this parasite is mainly restricted to juvenile birds, material was not readily available at all times of the year and therefore only two preliminary experiments have been possible to date.

Eggs extracted from specimens recovered from *Larus ridibundus* were allowed to develop at room temperature in water, and after about 7 days larvæ appeared. By the tenth day they had undergone ecdysis, the cast skin being retained as a sheath around each larva. Hatching commenced about 3 days later and continued for nearly 2 months. The larvæ both before and after hatching remained almost motionless and showed no migratory tendencies. In the first experiment approximately 150 eggs and larvæ which had undergone development for 30 days were administered to each of 4 one-month-old chickens. They were examined for helminths after 21–28 days, but none were found.

As the known hosts of *Cyathostoma lari* consume earthworms, *Lumbricus terrestris* was selected as a trial intermediate host for the second experiment. The earthworms were placed in sterilized soil enclosed in a polythene bag to reduce evaporation. About 50 *Cyathostoma* eggs which had developed for 23 days were pipetted into the gut of each of 22 earthworms. After 3 weeks the worms were fed to 11 three-week-old chickens, whilst a further 2 birds received eggs and larvæ direct. The birds were killed 21–28 days later. One adult female *C. lari* containing apparently maturing eggs was recovered from the orbital sinus of a bird which had received earthworms. No helminths were found in any of the other birds.

The life-cycle had thus been carried out experimentally in 54 days after recovering fertile eggs from the original female. While the shortest time required for completion of the life-cycle has not been established, it may well be less than this as Romanova[4] found that *Cyathostoma* sp. in the trachea of the emu reached sexual maturity in 21–28 days.

Cyathostoma lari has never been recorded from domestic chickens, and although the experiments reported here were only partially successful they demonstrate that the domestic fowl can be infested, perhaps only at an early age. Further work is necessary to elucidate the complete life-cycle of *C. lari*, which may be similar to that of its close relative, the gapeworm *Syngamus tracheæ*; that is, direct from bird to bird of the same species but requiring an intercalary host for transmission from wild birds to domestic poultry[5].

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Top Growth of Cultivated Tea

In India, tea bushes (*Camellia sinensis L.*) are pruned and plucked to a flat horizontal surface[1] the area of which can be easily estimated[2], provided that the bushes are sufficiently far apart without mutually interlocking frames. This condition was maintained in a field-trial in which 12 disparate populations were grown in the sun and under *Albizzia chinensis* trees, both with and without ammonium sulphate at the rate of 112 kgm. nitrogen per hectare (100 lb. per acre) per annum. The bushes were planted on the triangular system, 1·37 m. (4½ ft.) apart. The surface area of the bush frame was measured immediately after the annual pruning when the bushes were 15 years old.

The populations vary from wild-type var. *assamica*, through various intergrades, to populations with some features of the type variety (var. *sinensis*). As entities, all the populations could be assigned to var. *assamica*, but the range of variation is sufficient to suggest that our observation may be applicable to most tea cultivated in India that does not show marked attributes of the China or type variety.

Results of the experiment is given in Table 1 in terms of surface area of the bushes, yield of plucked shoots per unit area, and top growth removed from a unit of surface area. By top growth is meant the total green weight of prunings and pluckings. The analyses refer to growth made in the twelve months preceding the area measurement.

Table 1. *Camellia sinensis* var. *assamica*. Analysis of variance of bush area, yield per unit of area, and top-growth per unit of area

Source of variance	*D. F.*	Mean square		
		Area	Yield	Top-growth
Between block pairs				
Replicate, *J* × *N*	2, 3	0·050	0·168	1·069
Within block pairs				
Shade trees (*S*)	1	2·268**	1·935**	5·895**
J × *S* × *N*	3			
Error	2	0·060	0·092	0·337
Ammonium sulphate (*N*)	1	2·436***	0·408***	5·104***
Populations (*J*)	11	0·113***	0·048***	0·155
S × *N*	1	0·864***	0·016	1·964***
J × *S*	11	0·100	0·012	0·124
J × *N*	11	0·008	0·008	0·040
J × *S* × *N*	11	0·015	0·016	0·145
Error	86	0·014	0·012	0·150
Total	143	—	—	—

Bush area is expressed in sq. m., yield and top-growth in kgm. per sq. m. Significance at 5, 1 and 0·1 per cent levels are indicated by one, two and three asterisks respectively.

Top growth per unit area of bush surface is statistically the same for all populations showing that when the bushes are spaced sufficiently far apart for the differential development of their frames (Table 1), then the green weight of top growth removed from a unit area of ground covered by the frames is a constant for all populations. On the same basis, yield of plucked shoots which is the commercially important fraction of the top growth, differs between populations. This shows that a partition of growth between prunings and pluckings is a factor of importance in the agronomics of tea.

Further details of the crop yield of disparate populations will be given in a forthcoming publication[3] by Dr. W. Wight, to whom the author is indebted for the data presented in this communication.

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ENTOMOLOGY

Survival of Grasshopper Eggs Kept for Long Periods in Mineral Oil

The ease with which diapause may be broken in the eggs of a common North American grasshopper, *Melanoplus differentialis differentialis* (Thomas), with mineral (paraffin) oil has been described recently[1]. Treatment with the oil for 1 hr. or less is sufficient to remove the waterproofing materials from the hydropyle. After the eggs are taken from the oil, cleaned and transferred to a medium from which they can absorb water, development begins and they hatch in a completely normal manner about 18 days later. No harmful effect of the oil on the eggs can be detected even if they are left in it for as long as four weeks.

After the series of experiments reported in 1958 had been completed, 153 eggs from a single pod laid 15 days earlier and incubated at 25° C. since that time were dried briefly, placed in a Petri dish containing a thin film of mineral oil and returned to the incubator. The chorion was left intact. Six months later 103 of these eggs were selected and the chorion removed from each. They were freed from oil and placed to incubate on cotton-wool moistened with Ringer's solution which had been diluted with an equal volume of distilled water. During the three weeks which followed nine eggs spoiled and three were lost accidentally. All but one of the remaining eggs completed blastokinesis and 74 of them hatched. The nymphs behaved and grew to maturity exactly as do grasshoppers which develop in eggs kept under ordinary conditions. The percentage which hatched and that which completed nymphal development both lie well within the range commonly obtained when eggs are incubated in damp sand for six months at 25° C. Metabolic activity proceeds at a reduced rate when development is suspended[2]; but the supply of yolk gradually diminishes[3] and after six months the amount remaining in a particular egg may be insufficient to produce a viable nymph. In addition, waste products accumulate and the fat body becomes heavily laden with urate crystals.

When the eggs were examined after having been in mineral oil for six months it was observed that a light growth of fungus had appeared on the chorion of some of them and that a sharp, rancid odour reminiscent of butyric acid was noticeable. The 50 eggs left after the removal of the group of 103 were placed in fresh mineral oil and transferred to a clean container to discover whether any would survive an even longer period in the oil. Similar transfers were made at approximately 4-week intervals thereafter but mould re-appeared following each transfer and it seemed probable that all of the eggs would be killed. During the second 6-month period, 12 of the eggs showed obvious signs of decomposition and were discarded. At the end of one year in the oil the chorion was removed from each of the remaining 38 eggs and all were cleaned and placed to incubate on moist cotton. Within a few days rhythmic movements of the lateral body walls proved that the embryos in 20 of the eggs were still alive and that development had been resumed. Fourteen of these completed blastokinesis and seven of them hatched. Seven others completed embryonic development but failed to hatch. The rest died earlier. As this is written the first grasshopper to hatch is a vigorous sixth instar nymph. If some effective means of stopping the growth of mould had been employed it seems possible that a considerably larger number of eggs would have survived and hatched.

Since it has now been demonstrated: (1) that the eggs of this grasshopper may endure immersion in mineral oil for six months with a decrease in viability no greater than that encountered when eggs are kept under optimal conditions for a similar period and (2) that some eggs may even survive a full year in the oil, we now have even stronger evidence than we had in 1958 that the breaking of diapause in this species with wax solvents is not the result of injury to the tissues, as was claimed by Andrewartha[4] and Hinton[5].

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Forecasting Locust Outbreaks

LOCUST infestations are characterized by violent fluctuations in size; the red locust, *Nomadacris septemfasciata* (Serville), in a self-contained, controlled, outbreak area has been shown to vary by a factor of over 750 times[1,2]. However great the effects that predators and parasites may have, they do not prevent these fluctuations and in fact the only factors that depress a population and, at the same time, vary directly in strength with the size of the population, appear to be the emigration of swarms[3] and, nowadays, control with insecticides[4]. One therefore turns to weather as a non-density-dependent factor causing or permitting the large fluctuations, for there is no question of any spontaneous rhythm[4].

A search of published information and local records regarding two outbreak regions of the red locust, the Mweru wa Ntipa in Northern Rhodesia and the Rukwa Valley in Tanganyika, suggested that wet periods of years were always unfavourable to locust numbers and dry periods were sometimes favourable[5,6]. A similar suggestion had been made for *Locusta migratoria* (Linn.) in the Philippine Islands[7], China[8], and Russia[9,10], and for grasshoppers in the United States and Canada, mostly *Melanoplus* species (literature in ref. 11). Accordingly a careful investigation of data was made for that outbreak area in the Rukwa Valley for which the best data were available, namely the North Rukwa[12].

The red locust begins breeding with the start of the rains about November and the adults have all appeared when the rains are ending in April. The correlation with the success of breeding that was found was not so much the amount of rain in the same breeding season, as the amount of rain in the *previous* season, and the regression was negative and highly significant; a regression equation relating parental and filial populations to the previous season's rainfall and also to the amount of rain at the time of egg-laying in the current year gave a multiple correlation of $R=0{\cdot}93$ ($P<0{\cdot}01$). Although the exact mechanism has not yet been discovered, the forecasting that can be done with the aid of the equation has a very high cash value[12]. It means that by July of one year, when the rain records for the year become available, the numbers of hoppers in the following new year and the numbers of adults (if uncontrolled) one year later can be forecast. Consequently, supplies of insecticide, food and petrol, and numbers of men to be employed can be arranged well in advance without the risk of not having enough (with costly last-minute increases) or the waste of having too much.

This seems too good to be true; but there is general supporting evidence. First, the forecasting system has been successfully used in the North Rukwa for three successive years. Second, Lea[13] in South Africa has found a similar sort of relation between the previous year's rain and changes in population of the brown locust, *Locustana pardalina* (Walker). Third, a similar and almost equally reliable regression equation has been worked out by one of us (P.M.S.) for the red locust in the Malagarasi outbreak area. The locust infestation in a year (Y) is measured by the number of sectors in which locusts were found (scored one each) plus the number of sectors with bands or swarms (scored two each). The parental population (X_1) is assumed to have been proportional in the past to the number of adults earlier in the same year. The rainfall over the outbreak area and its drainage basin is assumed to be proportional to the maximum height achieved in a year by the Malagarasi River (X_2). (For information on river-levels we are indebted to Mr. Truss, manager of the Nyanza Salt Mines at Uvinza, and Mr. D. Pane of East African Railways and Harbours, Tabora). The Malagarasi River is the only drainage outlet of the region. It is not subject to rapid changes in height, because it is fed by flood-plains, including those which constitute the outbreak areas, and its maximum level in a year, which is reached in June, is probably a very good indication of the amount of rain received directly or indirectly in the outbreak area in the most recent season. Records for ten years have been analysed: during this period the largest locust infestation scored was 14 and the smallest 2. The independent regression of locust infestation on the criterion of parental infestation is positive and highly significant (regression coefficient $=0{\cdot}75$, $P<0{\cdot}01$) and on the maximum height of the Malagarasi River in the previous year negative and significant (regression coefficient $=-0{\cdot}58$, $P=0{\cdot}01$). The multiple regression is highly significant ($R=0{\cdot}89$, $P<0{\cdot}01$) and the equation, stated below, gives an error of estimate of ± 2:

$$Y=5{\cdot}6+0{\cdot}68\,X_1-0{\cdot}035\,X_2$$

(This analysis used the East African Railways and Harbours gauge figures though the Nyanza Salt Mines data give almost identical results; the figure required is the number of inches above 3,455 ft. that the river achieves at its maximum in a year.)

Schwerdtfeger[14] has put forward considerations which make what we have found more credible. If the size of a population of animals is determined by a large number of factors, favourable and adverse, none of which is individually outstandingly large in its effects, the probability of a large fluctuation in a particular year is not high; if, on the other hand, a population exists in generally favourable conditions, such as outbreak areas are now supposed to provide, and unfavourable factors are few and powerful, then large fluctuations may be expected. Contrariwise, if large fluctuations occur frequently, one may reasonably expect to find that a few factors are very important. The extreme case arises when one factor is almost over-riding; fluctuations are then likely to exhibit the violent character seen in outbreak areas of the red locust.

The only other species of locust in continental Africa to which outbreak area control can be fully and very economically applied is *Locusta migratoria migratorioides* R and F, which has its outbreak areas in the flood plains of the Middle Niger in the République Soudanaise. There, flooding is due partly to local rain and partly to rain falling hundreds of miles away in the hills at the Niger sources. Simple

inspection of the flood and rain records does not reveal an obvious correlation with populations of *Locusta* there, and the population does not seem to fluctuate very rapidly, but a proper investigation might give valuable results. In the same (Niger) area, there have now been three successive years of locally low rainfall and there has just been the largest upsurge of red locusts ever recorded there.

Forecasting of red locust populations is only one of the new techniques in use in the International Red Locust Control Service. It is linked with operational researches by J. H. Lloyd and W. N. Yule[1,15] which have revolutionized control, and with quantitative assessment of populations developed by C. C. Scheepers and others[2,16]. The result is a spontaneous non-Parkinsonian[17] reduction in costs, accompanied by a great increase in efficacy.

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Survival of Frozen Fat Body Cells in an Insect

Intracellular freezing is usually considered fatal to animal cells[1,2], whereas extracellular freezing can be tolerated if not too severe or prolonged[2,3]. The latter is well documented, but the former is based on negative evidence—the failure to obtain survival of cells known to have been frozen internally. Although mammalian red blood cells and spermatozoa are now commonly kept alive at very low temperatures by adding protective substances such as glycerol to the media, it is uncertain from the literature whether or not the cells themselves are frozen. Frozen mammalian tissue grafts have proved only partly viable, and it has been suggested that those cells that died may have done so because they were frozen internally[4].

In southern Alberta, larvæ of the goldenrod gall fly, *Eurosta solidaginis* (Fitch), hibernate above the snow during much of the winter, freezing and thawing many times without harm. The cells of the fat body are very large, 0·2–0·35 mm. in diameter, and contain many small colourless droplets of oil. Because of their large size, frozen fat cells were readily observed and manipulated in a cold oil bath (−15° to −25° C.) under a dissecting microscope. Observation was aided by injection of the larvæ with fast green dye in Ringer's solution, which coloured the hæmolymph but not the fat body. The following observations showed that the fat cells froze internally when the larvæ were supercooled and frozen as they would be in Nature. (1) Probing with a cold needle revealed the interior of the fat cells to be solid in the sense of being firmly granular or mealy. (2) Bits of cell contents raised to the warmer surface of the oil bath changed, upon melting, from a whitish, irregular mass to a light amber liquid sphere. (3) The fat cells retained their subspherical shape when frozen and, as nearly as could be judged, their original size, showing that no appreciable amounts of water were removed as would be expected if extracellular freezing alone had occurred.

Thawing after freezing causes many of the oil droplets to coalesce; if freezing has been severe or repeated, fusion of most of the droplets into one or two large globules occupying most of the cell produces a striking change in its appearance. (This 'globulation' has also been observed by the writer in the fat body of many other species and in the yolk of grasshopper eggs.) The size of the globules is roughly proportional to the severity of freezing, or to its repetition. *Eurosta* larvæ normally acquire globulated fat bodies during the winter, without detriment to their survival. To determine whether the condition persisted or was repaired during later development, larvæ were kept under observation after freezing. Preliminary assessments of globulation were made through the transparent cuticle on fat cells near the surface. No changes were seen up to the time that puparium formation and pigmentation obscured the view; thereafter puparia, pupæ, and adults were dissected, but at no stage were there changes in the size and numbers of oil globules. Hence globulation does not appear to affect the cells adversely in this species.

The possibility that the solidification and liquefaction within the fat cells was of lipid rather than aqueous nature was contra-indicated by the following observations. (1) When frozen fat cells and hæmolymph were slowly warmed together, they melted at the same temperature. (2) Large oil globules that had been formed by previous freezing and thawing could be detected in frozen tissue as colourless spheres. Cooling the oil to as low as −50° C., either *in situ* or after squeezing it out of the cells into hæmolymph, did not solidify the oil or make it less transparent.

It is apparent from these observations that the fat cells of cold-hardy *Eurosta* larvæ customarily freeze internally without harm to themselves or to the larvæ, despite the physical disruptions caused by freezing, melting, and the coalescence of their oil droplets.

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BACTERIOLOGY

Transduction in *Pseudomonas aeruginosa*

Transduction has been demonstrated for *Salmonella* with phage *P22*[1], for *Escherichia coli* with phage *P1*[2] and phage 363[3] and a transduction-like process has been described for *Pseudomonas aeruginosa*[4]. Bhaskaran[5] has described a process of genetic recombination in *Vibrio cholerae* which apparently involved transfer of genetic material by a bacteriophage.

In these experiments, a phage, *P110*, active on strain 1 of *Pseudomonas aeruginosa*[6], has been shown to transduce various auxotrophic markers to that strain of *Pseudomonas*. The phage was propagated on a donor prototrophic strain of 1 and allowed to infect various auxotrophic mutants of this strain. The transduction was detected by the production of prototrophs.

Phage *P*110 was isolated from a lysogenic strain of *Pseudomonas aerugomosa* and the procedures used in handling this phage were essentially the same as those described by Adams[7]. It forms well-defined plaques on strain 1 and high titres (10^9–10^{10}) can readily be obtained by the agar layer method of propagation.

The general procedure for the transduction experiments is closely parallel to that of Demerec *et al.*[8] for *S. typhimurium*. The acceptor auxotrophic strain was grown overnight at 37° C. on a shaker in Difco nutrient broth containing 0·5 per cent of added Difco yeast extract. The phage was propagated on the donor organism using the agar layer method with this same medium to which 10^{-3} *M* calcium chloride had been added. The bacterial culture was centrifuged and resuspended in chilled saline to a bacterial density of $2–5 \times 10^9$ organisms per ml. One ml. of bacterial suspension was mixed with 1 ml. of a phage preparation to give a multiplicity of from 5–10 and adsorption was allowed to take place for 30 min. at 37° C. The mixture was then spun down and resuspended in 2 ml. of chilled saline and 0·2 ml. plated on minimal agar plates. Colony counts were made after 48 hr. incubation at 37° C. Control cultures of the phage preparation and the auxotrophic suspension were included in all experiments to demonstrate absence of prototrophic contaminants in the phage and to measure back-mutation in the bacterial suspension.

A series of experiments of this type selecting for a number of auxotrophic markers gave regular but low frequencies of transduction. A marked limitation inherent in these experiments was the low (1–10 per cent) survival of bacteria following phage infection and means were sought for increasing the survival rate in the hope that this would increase the transduction frequency. Garen and Zinder[9] showed that irradiation of phage *P*22 with large doses of ultra-violet light decreased plaque formation without lowering the transducing ability. Application of this principle to *P*110 has yielded satisfactory results. Irradiation of phage lysates was carried out using a Westinghouse 17*W* Sterilamp. A dose of 10 min. at 50cm. reduced the titre of plaque-forming particles from 1×10^{10} to 2×10^8. Transduction experiments carried out with this irradiated phage showed a significant rise in transduction frequency when compared with transduction by unirradiated phage.

Table 1. Transduction by Irradiated and Non-Irradiated Phage

System	Prototrophs/10^9 recipient bacteria
Ps. aeruginosa strain 1 (met⁻) (methionine requiring) + Unirradiated *P*110 phage	16
Ps. aeruginosa strain 1 (met⁻) + Irradiated *P*110 phage	165
Ps. aeruginosa strain 1 (met⁻) + Broth	0

Unirradiated phage contained 1×10^{10} plaque forming particles /ml. Irradiated phage contained 2×10^8 plaque forming particles /ml.

Transduction was demonstrated also with four other auxotrophs of *Ps. aeruginosa* (Table 2). To date no linked transductions have been observed, and only one marker is transduced at a time.

Table 2. Transduction of Various Auxotrophic Markers by Irradiated Phage *P*110

Acceptor Auxotroph	Prototrophs/10^9 recipient bacteria
1 (ilva⁻)—isoleucine + valine requiring	122
1 (try⁻)—tryptophane requiring	20
1 (leu⁻)—leucine requiring	122
1 (ser⁻)—serine requiring	74

Back mutation-rates to prototrophy of all the auxotrophic markers are not significant.

The conclusion that *P*110 is responsible for the transfer of genetic material in the above experiments rests on the additional evidence that crystalline deoxyribonuclease does not inhibit the production of prototrophs, and specific anti-phage serum inhibits the production of prototrophs.

There is no connexion between this transduction and the sexual recombination previously reported in *Ps. aeruginosa*[6]. The strains used in all these experiments were *FP*–, and *FP*– × *FP*– crosses are always sterile[10].

It is seen that the genetic transfer carried out by phage *P*110 with strain 1 of *Ps. aeruginosa* parallels the action of *P*22 in *Salmonella* and *P*1 or 363 in *E. coli* and serves to underline the probable generality of the transduction mechanism.

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Metabolism of Cinnamic Acid and Hydroxy-Cinnamic Acids by *Lactobacillus pastorianus* var. *quinicus*

Previous work has shown that caffeic acid, 3 : 4 dihydroxy-cinnamic acid is metabolized by *Lactobacillus pastorianus* var. *quinicus*[1,2] with the formation of dihydrocaffeic acid and ethyl catechol[3]. A pure culture of this organism was inoculated into a basal medium[1] with the addition of 0·025 per cent cinnamic acid and 0·25 per cent fructose. After seven days' incubation at 25° C. under conditions of reduced oxygen tension, the cinnamic acid was completely metabolized. The end-product, an acid, was obtained as prismatic crystals, m.p. 47–48° C. in 85 per cent yield. A mixed melting-point with an authentic specimen of dihydrocinnamic acid showed no depression. Elementary analysis (Drs. Weiler and Strauss) gave C 71·85 per cent, H 6·84 per cent; $C_9H_{10}O_2$ require requires C 72·0 per cent, H 6·7 per cent. The yield indicated that the organism had reduced cinnamic acid to dihydrocinnamic acid with little or no subsequent decarboxylation.

Inoculation of the organism into a similar medium containing 0·025 per cent *p*-coumaric acid (*p*-hydroxy-cinnamic acid) and 0·25 per cent fructose followed by incubation under the same conditions resulted in complete metabolism of the *p*-coumaric acid. Two products, *A* and *B*, were obtained and separated on a silica gel column. *A* had R_F 0·72 in 2 per cent acetic acid and R_F 0·42 in *n*-propanol—conc. ammonia (70 : 30); spray reagents on paper chromatograms reacted as follows: (1) 1 per cent ferric chloride—1 per cent potassium ferricyanide (blue colour); (2) 0·1 per cent ethanolic 2 : 6 dichlorophenolindphenol (red colour on a blue background); (3) diazotized *p*-nitraniline followed by sodium carbonate solution (purple colour); (4) diazotized sulphanilic acid

followed by sodium carbonate solution (red colour). Phloretic acid (*p*-hydroxy-phenylpropionic acid) showed the same colour reactions and had identical chromatographic properties. *A* had a melting-point of 128–129° C. and admixture with an authentic specimen of phloretic acid gave no depression; *A* was therefore identified as phloretic acid.

B streaked in 2 per cent acetic acid but ran just behind the solvent front in *n*-propanol- conc. ammonia (70 : 30); spray reagents on paper chromatograms gave results as follows: (1) 1 per cent ferric chloride—1 per cent potassium ferricyanide (blue colour); (2) 0·1 per cent ethanolic 2 : 6 dichlorophenolindophenol (no reaction); (3) diazotized *p*-nitraniline followed by sodium carbonate solution (yellow colour); (4) diazotized sulphanilic acid followed by sodium carbonate solution (red colour). These results suggested that product *B* was *p*-ethyl phenol, a decarboxylation product of phloretic acid. *B* was obtained as white needle-like crystals from petroleum ether (B.P.) 40–60° C. with a melting-point of 45·5° C.; admixture with an authentic specimen of *p*-ethyl phenol gave no depression of the melting-point. *B* was therefore identified as *p*-ethyl phenol.

Lactobacillus pastorianus var. *quinicus* is therefore able to reduce the side-chain of cinnamic acid, 4 : hydroxy and 3 : 4-dihydroxy-cinnamic acids. The products may then be decarboxylated, the extent of decarboxylation decreasing as the number of phenolic hydroxyl groups in the molecule decreases; dihydrocaffeic acid yielded 90 per cent ethyl catechol, phloretic acid gave 60 per cent *p*-ethyl phenol while dihydrocinnamic acid could not have been decarboxylated to an amount of more than 15 per cent.

Reduction of the cinnamic acid side-chain may be widespread; soil *Pseudomonas* spp. under aerobic conditions convert cinnamic acid to 2-hydroxyphenylpropionic and 2 : 3-dihydroxyphenylpropionic acid[4]; rats, rabbits[5] and man[6] are able to reduce caffeic acid to dihydrocaffeic acid and ferulic acid (3-methoxy-4 OH cinnamic acid) to dihydroferulic acid (3-methoxy-4-hydroxyphenylpropionic acid). A comparison may also be made with the reduction of acrylic acid to propionic acid by the rumen organism *LC*[7]. Decarboxylation of the phenylpropionic acid side-chain, as brought about by *Lactobacillus pastorianus* var. *quinicus*, has however not been reported in other bacteria or in animal tissues.

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Incorporation of Molecular Oxygen in Bacterial Cells Utilizing Hydrocarbons for Growth

The methane-utilizing bacterium *Pseudomonas methanica* oxidizes ethane to ethanol, acetaldehyde and acetic acid; propane to *n*-propanol, propionic acid and acetone; and *n*- butane to *n*-butanol, *n*-butyric acid and 2-butanone[1]. The formation of homologous oxygenated products made possible a study of the mechanism of the bacterial oxidation. Ethylene could not be detected as an intermediate during the conversion of ethane, nor was ethylene converted to the same products as ethane. Mass spectrometric analysis of the acetic acid and the acetaldehyde produced from deuteroethane (ethane-D_6) eliminated the possibility that ethane was dehydrogenated to ethylene at a stage in the oxidation[1].

The possibility of bacterial oxygenation with molecular oxygen[2] has been studied. Indirect evidence for such a mechanism already exists in the case of another bacterium utilizing hydrocarbon[3]. The various volatile oxygenated compounds were produced by *P. methanica* in the presence of an atmosphere containing oxygen-18. They were isolated and analysed for isotope content. No significant enrichment of oxygen-18 was found in any of the oxidation products. Results from control chemical experiments employing $H_2{}^{18}O$ made it highly probable that if the bacterial products initially contained oxygen-18, exchange reactions would preclude its presence in significant amounts when analysed.

Evidence for incorporation of molecular oxygen in bacterial cells during hydrocarbon utilization was obtained in growth experiments with three different bacteria utilizing hydrocarbons. Each organism listed in Table 1 was grown in mineral-salts solution

Table 1

Organism	Carbon source	Atom percentage excess oxygen-18		Percentage of cell material oxygen derived from O_2
		O_2	Cell material	
Pseudomonas methanica	Methane (50 per cent v/v)	6·2	0·32	5·1
	Methanol (0·5 per cent w/v)	6·2	0·02	0·32
"Propane bacterium"	Propane (50 per cent v/v)	6·0	0·16	2·8
	Tridecane (0·67 per cent w/v)	6·0	0·10	1·8
	Glucose (0·5 per cent w/v)	6·0	0·05	0·83
"Ethane bacterium"	Ethane (50 per cent v/v)	6·2	0·44	7·1
	Propane (50 per cent v/v)	6·2	0·22	3·5
	n-Butane (30 per cent v/v)	6·2	0·10	1·8
	Glucose (0·5 per cent w/v)	6·2	0	0

'Propane bacterium' and 'ethane bacterium' are unidentified species isolated from propane and ethane enrichment cultures, respectively. The propane bacterium is a Gram-variable, acid-fast rod ; the ethane bacterium is a Gram-positive, non-acid-fast rod. Cultures consisted of 150 ml. of mineral salts solution[4] in gas-tight 1-l. side-arm flasks. The atmosphere in each case contained 10 per cent oxygen. Incubation temperature was 30° C. for *P. methanica* and the ethane bacterium ; 37° C. for the propane bacterium. Cultures were shaken continuously for 3–5 days. Hydrocarbons were 'Pure Grade', at least 99 mole per cent purity, purchased from Phillips Petroleum Company, Bartlesville, Oklahoma.

with an individual hydrocarbon as the sole source of carbon and energy in an atmosphere containing oxygen-18. As a control, each organism was also grown on a suitable non-hydrocarbon substrate in a similar atmosphere. At maximal growth the cultures were harvested by centrifugation, washed in distilled water, lyophilized and the dry cell matter converted to carbon dioxide by the method of Ritterberg and Ponticorvo[4]. The oxygen-18 composition of the various specimens of carbon dioxide and of the oxygen initially present in the culture vessles is given in Table 1. The results show that cells produced from each of the hydrocarbons had decidedly higher contents of oxygen-18 than the cells derived from the non-hydrocarbon substrates. The results are remini-

scent of the oxygen-18 enrichment of cells of *Pseudomonas* sp. growing at the expense of aromatic acids[5].

Interesting differences were found in the oxygen-18 content of cells grown on different hydrocarbons. Thus, the 'propane bacterium' had a significantly lower content of oxygen-18 when grown on tridecane than on propane. The gradation is even more striking in the 'ethane bacterium'. Here, the oxygen-18 content of the cells was inversely related to the carbon chain length of the substrate hydrocarbons tested.

These experiments establish that hydrocarbon utilization by these bacteria is accompanied by incorporation of molecular oxygen. Differences in the oxygen-18 content of cells grown in the various hydrocarbons may be explained by the utilization pathways of the respective primary oxygenated hydrocarbon intermediate. Thus, assimilation of intermediates produced from substrates with chains longer than C_2 involves additional reactions in which the oxygen of water is utilized, the net effect being a dilution of the oxygen-18 of the cells.

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CYTOLOGY

Unimpaired Mitosis in Cells with Modified Deoxyribonucleic Acid

The phenomenon of mitosis confronts the investigator with mysteries which become more and more occult with the increase in our knowledge of the basic biochemistry of the nucleus. Although the intense current interest in this field has produced many hypotheses concerning the structure, function, mode of replication of deoxyribonucleic acid, and its relationships with ribonucleic acid and protein, much of this is based on circumstantial evidence.

Great effort has been expended in determining spatial relationships and measuring linkage distance in attempts to define ultra-structure, in the belief that knowledge of the ultra-structure of nuclear material will be of ultimate importance in arriving at an idea of the ultra-function of the nucleus. A corollary of this view is that alteration of this structure will produce corresponding changes in nuclear function. For example, it has been believed that cells which admit dye to the nucleus are dead or dying[1].

Some recent reports have indicated that the acridines, which are nuclear dyes, do not harm cells[2,3]. Living intact organisms were used in these short-term experiments, however, and it has been shown in sarcoma 180 ascites tumour *in vivo*[4], and confirmed by us in Ehrlich ascites tumour *in vivo* and fibroblasts *in vitro*, that the binding of acridine orange to the nucleus is highly reversible when the extracellular dye concentration is not maintained.

Other workers have reported alterations in cell

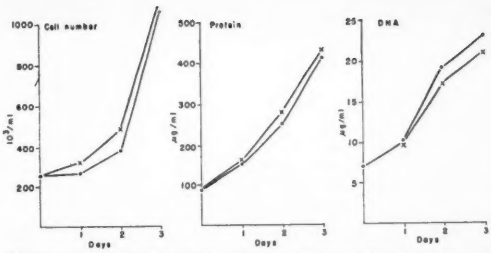

Fig. 1. Growth of L-cells in culture with and without acridine orange. 150 μgm./100 ml. of acridine orange were added to one set of suspension cultures, and these cultures were carefully shielded from light at all times. In all other respects, the paired cultures were treated identically. Deoxyribonucleic acid and protein were determined as previously described (ref. 12). The above values represent the average of at least 4 experiments. ●——●, Control cultures; ×——×, cultures treated with acridine orange

function after treatment of the cells with the fluorescent acridine dyes[5,6]; however, these reports contain no mention of shielding of the cells from light. In some of them, in fact, the cells were continuously observed under strong illumination[5]. We have found marked effects of light on living cells stained with acridine orange[7], and it is possible that the effects reported were not due directly to acridine orange, but were mediated through its photosensitizing action.

We have grown L-strain fibroblasts under continuous agitation[8] in the constant presence of acridine orange, kept at concentrations ranging from 15 to 150 μgm. per 100 ml. in various experiments. We have detected no appreciable effect under these conditions on rate of multiplication, replication of deoxyribonucleic acid, or protein synthesis (Fig. 1). Acridine orange being a potent photosensitizer[7], it was found necessary to shield the cultures from all light. The presence of acridine orange within the nuclei of all the cells in a given culture was regularly and unequivocally demonstrated at any given time with the aid of fluorescence microscopy. Aliquots of cells were removed from the cultures, kept on a glass slide in darkness and immediately observed with the fluorescence microscope.

Alternatively, cells were grown on glass slides without agitation; since these cells spread out on the glass, nuclear and cytoplasmic detail could be observed at a greater advantage. All stages of mitosis

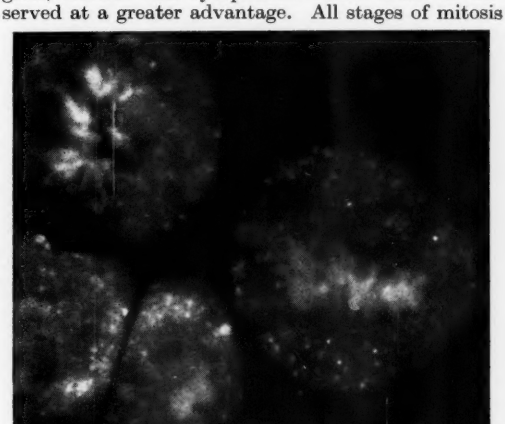

Fig. 2. Metaphases in living cells in culture. The photograph was taken with the aid of a Reichert fluorescence microscope, using a mercury vapour light-source filtered to transmit ultra-violet and blue light. Acridine orange is seen attached to the nuclear chromatin material, and in the cytoplasm as small particles. L-cells grown on glass slides in 75 μgm./100 ml. acridine orange. (×1000)

were observed, and the chromosomes were clearly stained with acridine orange (Fig. 2). These observations were not changed by washing the cells with saline. Similar results were obtained with Ehrlich ascites cells in culture and random pure cultures of bacteria.

Much evidence exists that acridine orange and other acridine dyes become attached to nucleic acid. *In vitro*, a strong affinity exists between nucleic acids and the acridine orange[9]. *In vivo*, nuclear staining with acridine orange has been felt by other authors to represent nucleic acid staining[2,10]. The dye is strongly basic (*pK* 10·1), whereas deoxyribonucleic acid has many acidic groups, and nucleoprotein is basic. In fixed tissues, the pattern of staining corresponds to the chromatin pattern, and prior treatment with ribose and deoxyribose nucleases abolishes this staining[11]. Using synthetic and natural polynucleotides, Beers *et al.* have presented evidence that the phosphate groups of nucleic acid, particularly the terminal phosphates, are the site of attachment of acridine orange[12].

If we can accept this hypothesis that acridine orange does become attached to deoxyribonucleic acid, then we can reasonably suggest that this results in loss of spatial integrity of the deoxyribonucleic acid molecule. Our observations show clearly that this change does not result in alterations in cell division, deoxyribonucleic acid replication or protein synthesis.

It is hoped that further experiments will reveal some parameter of cellular function which is sensitive to the acridine dyes: perhaps some information concerning the intimate function of deoxyribonucleic acid in the cell may be thus uncovered.

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FORESTRY

Limitations Imposed by some Water-borne Preservatives on the Soil Block Test Method for Timber

When loss of weight is used as the criterion for fungal attack of treated blocks of timber tested by the soil block method the precise value of the threshold concentration of preservative is often obscured by extraneous losses of weight in sound blocks.

With creosote and oil-solvent preservatives this loss is due to the volatilization of some of the preservative or carrier during the exposure period and as these losses are fairly constant they can be discounted when assessing the results.

With water-borne preservatives such losses are found only with some salts, and an investigation into the cause of this has shown it to be due to soluble salt removal by diffusion. For such diffusion to occur it is generally accepted that the moisture content of the treated blocks should rise above fibre-saturation point and this has been shown to occur when the preservative used does not become thoroughly fixed in the wood or when a soluble salt is formed as a by-product of the fixation reaction.

Table 1

Preservative	Dry salt retention (lb./cu. ft) 0	0·09	0·18	0·35	0·7	1·4
	Moisture Content (Percentage oven-dry wood weight)					
Copper-chrome-arsenate (oxides)	28	30	29	29	29	29
Copper-zinc-chrome-arsenate (oxides)	30	30	29	30	29	30
* Copper-chrome-arsenate ($CuSO_4$ & $K_2Cr_2O_7$)	29	32	37	41	43	62
Fluor-chrome-arsenate-phenol	29	—	34	43	62	92
Boric acid	31	30	32	34	38	79

* In this formulation the potassium and sulphate ions take no part in the fixation reactions and presumably remain in the wood as a soluble salt.

Table 1 shows moisture contents obtained in blocks treated with five commercial preservatives to retentions ranging from 0·09 to 1·4 lb. per cu. ft. dry salt and incubated in sterile soil jars for 4 weeks. (The moisture content of all blocks before incubation was 10 per cent.)

If the treated blocks are well leached with running water prior to incubation no moisture build-up occurs with any of the preservatives tested.

Further tests showed that this increase in moisture content was obtained over a wide range of soil moistures and that the greater part of the moisture taken up came from the atmosphere above the soil rather than by moisture absorption directly from the soil. Hygrometer readings in this atmosphere showed that full saturation is approached at all soil moistures above the equilibrium moisture content of the soil (2–3 per cent with the soil used) at the temperature of incubation and that it is maintained as long as there is any surplus moisture in the soil.

The diffusion of salt from the treated blocks into the feeder strips subsequent to moisture build-up can be readily seen with highly coloured non-fixed salts such as dinitrophenol and has been checked by chemical analysis in the case of boric treated blocks.

The implications of this phenomenon in relation to non-fixed, unleached preservatives are two-fold.

First each retention of each preservative creates its own micro-environment so that results obtained from these are not strictly comparable. In the extreme cases the moisture content can become so high that fungal development could be limited by lack of aeration alone.

Secondly, salts can be removed by diffusion from the treated blocks causing weight losses of unknown magnitude. This migration may also inhibit fungal development in the feeder strips and underlying soil.

A more detailed report of this study is being made and will be reported elsewhere.

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